10/019902
531 Rec'd PCT/PTO 21 DEC 2001

Intermolecularly associating compounds, and aggregates comprising them

The present invention relates to special low molecular weight compounds suitable for forming aggregates by intermolecular association. The present invention relates also to aggregates comprising such compounds, and to processes for the preparation of such aggregates. The invention relates also to special uses of the compounds and aggregates, especially for therapeutic and diagnostic purposes.

The simultaneous and specific association of at least two ligands with corresponding receptors results in multivalent interactions between two units carrying those ligands or receptors. Such multivalent interactions are very widespread in biology, it being possible for the interacting units to have ligands such as oligosaccharides, proteins, nucleic acids or lipids. Multivalent interactions are characterised by a large number of individual weak monovalent bonds which in biological systems are frequently preferred over a single strong monovalent bond (M. Mammen, S-K. Choi, G. M. Whitesides, *Angew. Chemie*, **110**, 2908, 1998).

In biological systems, multivalent interactions are frequently developed when bonds are formed between units with ligands and receptors having little affinity. Known examples of interactions between ligands and receptors having little affinity are carbohydrate-protein and carbohydrate-carbohydrate interactions (A. Danguy, K. Kayser, N. V. Bovin, H.-J. Gabius, *Trends Glycosc. Glycotech.*, **7**, 261, 1995), which, for example, in viral and bacterial infections play a crucial role in the onset of inflammatory processes, in the formation of tumour metastases or in immunorecognition.

Natural multivalent interactions can be blocked especially for therapeutic and diagnostic purposes. For the *in vitro* blocking of such multivalent interactions, both monovalent and multivalent inhibitors have been used hitherto.

In the case of derivatives of natural ligands as monovalent inhibitors it has been shown in practice that as a result of the low binding affinity it is not possible to achieve efficient inhibition of multivalent interactions. For example, the binding constant in the case of interaction between a monovalent galactoside and the corresponding lectin is only $K_D \sim 10^4$M (D. T. Connolly et al., *J. Biol. Chem.*, **257**, 939, 1982). For therapeutic use in such a case, very large amounts of inhibitor would have to be used. A method of treatment using such an inhibitor would not therefore be cost-effective.

Known multivalent inhibitors include those in which a plurality of ligands are covalently bonded to a low molecular weight carrier (L. L. Kiesling, N. L. Pohl, Chemistry & Biology, **3**, 71, 1996; G. D. Glick, P. L. Toogood, D. C. Wiley, J. J. Skehel, J. R. Knowles, J. Biol. Chem., **266**, 23660, 1991) or to a dendrimer (D. Zanini, R. Roy, *J. Org. Chem.*, **63**, 3486, 1998). In those cases, however, the specific binding affinity is only very slightly increased.

WO 98/14215 discloses glucoconjugates as inhibitors of viral cell adhesion. In particular, the compound [Neu5Acα2-6Galβ1-4GlcNAcβ1-NHCOCH$_2$NH-CO(CH$_2$)$_4$CO-(NHCH$_2$-CO)$_3$-NHCH$_2$-]$_4$C is disclosed. That compound does not, however, form aggregates in aqueous solution.

Also known are multivalent inhibitors in which the active ligands are bonded to a polymeric carrier. Such compounds exhibit increased efficiency in comparison with the corresponding monomeric ligands. By way of the example of the interaction between the influenza-haemagglutinin, which binds to neuraminic acid derivatives on the cell surface, it has been shown how the use of a polymer-based multivalent inhibitor affects that interaction (monovalent: $K_D \sim 2 \times 10^{-4}$M, multivalent: $K_D \sim 3 \times 10^{-7}$ M; A. Spaltenstein et al., *J. Am. Chem. Soc.*, **113**, 686, 1991).

Despite their improved effectiveness, the multivalent polymeric inhibitors known hitherto are also unsuitable for therapeutic use. The disadvantages are to be attributed to the polymeric carrier molecules used and to the properties thereof.

When polylysine or sulfated polysaccharides are used as polymeric carriers, non-specific ionic interactions with cell surface structures take place.

Polyacrylamides and other polymers, the polymer content of which consists exclusively of C-C bonds, have the crucial disadvantage that they are broken down in the organism to form toxic metabolites.

High polymers (60-70 kDa) are not effectively filtered by the kidneys and their breakdown by the liver can lead to intolerances as a result of the formation of toxic metabolites.

Patent applications EP 601 417 and WO 95/34673 describe polymer-based carbohydrate receptor blockers that are physiologically tolerable both in the form of the total molecule

and in the form of breakdown products. Those properties are achieved by the use of biodegradable polymers. For use as a medicament, however, those products too have a fundamental disadvantage because, in practice, polymers are not pure and precisely defined compounds, but rather consist of complex mixtures of compounds of different molecular size. This circumstance renders the use (approval) of such a polymeric inhibitor as a medicament extraordinarily difficult.

In the case of a medicament it is important to have accurate knowledge of the associations between the chemical structure of an active ingredient and its pharmacological properties. In the case of substance mixtures, it would have to be shown in what way the composition of a mixture influences its particular pharmacological properties. In addition, a medicament must be precisely defined in its chemical composition and must be demonstrably preparable in precisely that form. Neither requirement can be fulfilled in the case of the polymeric multivalent inhibitors using the synthetic and analytical methods currently available and using a technically sensible level of resources.

A further group of multivalent inhibitors comprises compounds wherein the ligands are bonded to the surface of liposomes. Liposomes have the disadvantage that their lipophilic constituents are able to enter into non-specific interactions, for example by being incorporated into cell membranes.

The problem underlying the present invention is therefore to avoid the disadvantages of the prior art and to make available new compounds having improved properties as multivalent inhibitors of biological recognition processes, the compounds having a specific action and being suitable for use as medicaments.

That problem is solved in accordance with the claims using a compound of the general formula (I)

$$X(B)_m \qquad (I)$$

wherein

X is an m-valent unit and

B are identical or different and denote K-R,

wherein

K is a bond or is A^1-(A^2-A^3)$_k$-sp, wherein

A^1 is $(CH_2)_tY(CH_2)_u$, wherein

Y is >C=O, >NH, -O-, -S- or a bond,

t is an integer from 0 to 6 and

u is an integer from 0 to 6,

A^2 is -NHCO-, -CONH-, -OCONH- or SCONH-,

A^3 is $(CH_2)_r$, $O(CH_2)_r$, $NH(CH_2)_r$, $S(CH_2)_r$ or -(CHQ)-, wherein

r is an integer from 1 to 6 and

Q is a substituted or unsubstituted alkyl or aryl group,

sp is a divalent spacer or a bond, and

k is an integer from 5 to 100, and

R is hydrogen; a ligand suitable for specific binding to a receptor; a marker molecule; or a catalytically active group; and

m is at least 2,

with the proviso that

(1) in the compound at least one R is not hydrogen,

(2) there are at least two K that are not a bond, and

(3) X, B and m are so selected that an intermolecular association of the K in liquid phase by the formation of hydrogen bonds is possible, with formation of aggregates that present on the surface a plurality of R that are not hydrogen, and

(4) the molar mass of the fragment $X(K)_m$ is less than 20,000.

In the compound of formula (I), A^2 may also be -CO-.

Further preferred embodiments are the subject of the subsidiary claims.

In a preferred embodiment, the molar mass of the fragment $X(K)_m$ is less than 10,000, preferably less than 4,000.

Self-association of compounds of the general formula (I) gives rise to aggregates that act as highly efficient multivalent inhibitors of biological recognition processes.

In the compounds of formula (I), X, B and m are so selected that an intermolecular association of the K in liquid phase is possible, especially under aqueous conditions,

preferably under *in vivo* conditions, with formation of aggregates that present on the surface a plurality of R that are not hydrogen.

It has been found that by the formation of the aggregates according to the invention the disadvantages of the previously known multivalent active ingredients can be avoided.

It has been found especially that the slight increase in binding affinity in comparison with a monovalent active ingredient where a plurality of ligands are covalently bonded to a low molecular weight carrier or to a dendrimer is to be attributed to the fact that although such molecules do present a plurality of ligands, the latter cannot be arranged (or only some of them can be arranged) in such a manner that a thermodynamically advantageous interaction with receptors is achieved. It has been found that the interaction of a polyvalent active ingredient can be improved by dynamically coupling the ligand arrangement to the receptor arrangement. It has been found that this dynamic coupling can be achieved by way of an intermolecular aggregate formation in which special molecular regions of the active ingredient associate intermolecularly and thus an adaptation of the ligand arrangement is facilitated. Finally, it has been found that the adaptation of the ligand arrangement so facilitated results in a drastic increase in the binding affinity of the polyvalent active ingredient.

By virtue of the reversibility of the aggregate formation, the compounds of the present invention enable a molecular unit to interact polyvalently with a plurality of receptors, with subsequent optimisation of the ligand arrangement, there being found a thermodynamically advantageous arrangement without any undesirable side effects, such as the insertion of the compounds into the cell membrane.

The compounds of the present invention are small molecules, which would not be expected to have an action as antigen, and the other disadvantages that occur with polymeric polyvalent active ingredients are also avoided.

The molecular structure of the compounds of general formula (I) is substantially distinguished by three structural features:

- an m-valent fragment X,
- a plurality of molecule chains K, which are covalently bonded to the fragment X,
- at least one terminal group R, which is a ligand suitable for specific binding to a receptor; a marker-molecule; or a catalytically active group.

The molecule chains K are distinguished by a chemical structure that allows an intermolecular association in liquid phase also under aqueous conditions, especially *in vivo* conditions, with formation of aggregates. The formation of the aggregates is based on non-covalent interactions, it being possible for the non-covalent interactions to be ionic interactions, van der Waals interactions, hydrophobic interactions or preferably hydrogen bonds. The structure of non-covalent bonds between a plurality of compounds of the general formula (I) brings about a self-association and thus the formation of aggregates.

The compounds of the general formula (I) have at least one terminal group R that is derived, for example, from a biologically active ligand or from a marker. The terminal groups R are covalently bonded to the terminal ends of the molecule chains serving for the association. The bonding of those groups can be effected directly or by way of a spacer. As spacer there can be used a divalent molecular fragment which does not participate in the intermolecular association brought about by non-covalent interactions, but which merely serves to hold the terminal groups R. Such a spacer is formally part of the molecule chain K.

According to the invention, K in the formula (I) may be

$$A^1\text{-}(A^2\text{-}A^3)_k\text{-}sp$$

wherein

A^1 is $(CH_2)_tY(CH_2)_u$, wherein
- Y is >C=O, >NH, -O-, -S- or a bond,
- t is an integer from 0 to 6 and
- u is an integer from 0 to 6,

A^2 is -NHCO-, -CONH-, -OCONH- or SCONH-,

A^3 is $(CH_2)_r$, $O(CH_2)_r$, $NH(CH_2)_r$, $S(CH_2)_r$ or -(CHQ)-, wherein
- r is an integer from 1 to 6 and
- Q is a substituted or unsubstituted alkyl or aryl group,

sp is a divalent spacer or a bond, and

k is an integer from 5 to 100.

In the compound of formula (I), A^2 may also be -CO-.

Special preference is given to compounds of the general formula (I), wherein

m is an integer from 2 to 4, and

X is CH_{4-m}, NH_{3-m}, N^+H_{4-m}, >P- (when m = 3), $>P^+<$ (when m = 4), >B- (when m = 3), a linear atom group C_2H_{6-m}, $>CH(CH_2)_zCH<$, >C=C<, >N-N<, $>N(CH_2)_zN<$ wherein z = 2 - 6, when m = 4), a carbocyclic atom group C_6H_{6-m}, C_6H_{12-m}, or a heterocyclic atom group C_3N_3 (when m = 3), C_4N_2 (when m = 4).

It is especially preferable for at least 3 K to be present in a compound of the general formula (I). Special preference is given to compounds of the general formula (I) in which at least two R, preferably three R, are not hydrogen.

When more than one terminal group R is present in a compound of the general formula (I), those groups may be identical or different.

As examples of the ligands suitable for specific binding to a receptor that function as terminal groups R of compounds of the general formula (I) there may be mentioned naturally occurring biological recognition structures, such as mono- or oligo-saccharides, peptides, mono- or oligo-nucleotides or nucleic bases. It is also possible, however, to use synthetic derivatives of those compounds or other organic or inorganic compounds that are recognised by biological receptors. As ligands there may also be used known compounds that are used in free form as therapeutic active ingredients. There may be mentioned by way of example:

- anti-tumour agents, such as, for example, daunomycin, doxorubicin, vinblastine, bleomycin;
- antibiotics, such as, for example, penicillins, erythromycins, azidamfenicol, cefalotin and griseofulvin;
- antagonists of blood platelet activation factors;
- leucotriene antagonists;
- inhibitors of the cyclooxygenase system, such as, for example, salicylic acid compounds;
- lipoxygenase inhibitors;
- antiphlogistics, such as, for example, indometacin;
- antirheumatics, such as, for example, nifenazone;
- therapeutic radionuclides, such as, for example, bismuth;
- neuraminidase;

- inhibitors, such as, for example, zanamivir.

It is preferable to use oligosaccharides that are present on cell surfaces as constituents of glycoproteins, glycolipids or proteoglycans, and also any desired constituent parts thereof.

Special oligosaccharides that can are be used as terminal group R are as follows: sialic acid, sialyl lactose, sialyl lactosamine, lactose, Galα1-3Gal, Galα1-3(Fucα1-2)Gal, GalNAcα1-3(Fucα1-2)Gal, Neu5Acα2-6GalNAc, SiaLe^A, SiaLe^X, HSO_3Le^A, HSO_3Le^X, Galα1-3Galβ1-4GlcNAc, Galα1-3Galβ1-4Glc, Neu5Acα2-6Galβ1-4GlcNAc.

In addition, preference is given to sialic acid benzyl glycoside, HSO_3GlcAβ1-3Gal, HSO_3GlcAβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc, GalNAcα, GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAc, Galα1-3(Fucα1-2)Galβ1-4GlcNAc, HSO_3(Sia)Le^X, HSO_3(Sia)Le^A, Le^Y, GlcNAcβ1-6(GlcNAcβ1-3)Galβ1-4Glc, GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glc, mannose-6-phosphate, GalNAcβ1-4GlcNAc, oligo-sialic acid, N-glycolylneuraminic acid, Galα1-4Galβ1-4Glc, Galα1-4Galβ1-4GlcNAc.

Derivatives or mimetics of the above-mentioned mono- or oligo-saccharides, peptides, mono- or oligo-nucleotides or nucleic bases can also be used.

The terminal groups R can also be derived from marker molecules. Such marker molecules enable compounds of the general formula (I) to be used in diagnostic applications. All marker molecules known to the person skilled in the art for *in vitro* diagnostic test systems, such as, for example, biotin, fluorescein, rhodamine, digoxigenin or radioactive markers, come into consideration for the purposes of the present invention. Special mention may be made of markers known to the person skilled in the art for *in vivo* diagnosis, such as radioactive markers that contain a bound radionuclide, e.g. technetium, X-ray contrast media that contain e.g. an iodised compound, or nuclear resonance contrast media, e.g. based on gadolinium compounds.

It is proposed that in a preferred embodiment the terminal groups R be so selected that aggregates are obtained which, on the one hand, interact with suitable receptors by way of suitable ligands through polyvalent interactions and, on the other hand, contain marker units. As a result, the polyvalent interactions are accessible to detection and the compounds can be used in a diagnostic procedure.

The aggregates can in this case be synthesised from compounds of formula (I) that contain both ligands and the marker radicals. Such an aggregate preferably comprises only one special compound of the general formula (I). On the other hand, however, an aggregate can also comprise a plurality of different compounds of formula (I), the compounds containing either ligands or marker radicals.

The present invention also provides an aggregate of the following general formula (II)

$$\{X(B)_m\}_n \qquad (II)$$

wherein

$X(B)_m$ may be identical or different and denote a compound of the general formula (I), as defined in any one of claims 1 to 11, and

n is from 2 to 100,000,

and wherein $X(B)_m$ are non-covalently bonded.

The present invention provides especially an aggregate having a leaf-like structure and having linear, cyclic, polycyclic, polyhedral, spherical or dendritic structure. The aggregates may consist of two or more different compounds of the general formula (I).

The present invention also provides compounds of the general formula (III). The compounds of general formula (III) correspond to those of formula (II) wherein all terminal groups R are hydrogen atoms. Such compounds can be used with the compounds of the general formula (I) described above in order to alter the properties of the aggregates.

The present invention provides especially a compound of the general formula (III)

$$X(B)_m \qquad (III)$$

wherein

X is an m-valent unit and

B are identical or different and denote K-H,

wherein

K is A^1-(A^2-A^3)$_k$-sp,

wherein

A^1 is $(CH_2)_tY(CH_2)_u$, wherein
Y is >C=O, >NH, -O-, -S- or a bond,
t is an integer from 0 to 6 and
u is an integer from 0 to 6,
A^2 is -NHCO-, -CONH-, -OCONH- or SCONH-,
A^3 is $(CH_2)_r$, $O(CH_2)_r$, $NH(CH_2)_r$, $S(CH_2)_r$ or -(CHQ)-, wherein
r is an integer from 1 to 6 and
Q is a substituted or unsubstituted alkyl or aryl group,
sp is a divalent spacer or a bond, and
k is an integer from 5 to 100, and

m is at least 2,

with the proviso that

(1) X, B and m are so selected that an intermolecular association of the K in liquid phase is possible, especially under aqueous conditions, by the formation of hydrogen bonds, with formation of aggregates, and

(2) the molar mass of the fragment $X(K)_m$ is less than 20,000, especially less than 4000.

In the compound of formula (III), A^2 may also be -CO-.

In a preferred embodiment, K in formula (III) is

$$A^1\text{-}(A^2\text{-}A^3)_k\text{-}sp$$

wherein

A^1 is $(CH_2)_tY(CH_2)_u$, wherein
Y is >C=O, >NH, -O-, -S- or a bond,
t is an integer from 0 to 6 and
u is an integer from 0 to 6,
A^2 is -NHCO-, -CONH-, -OCONH- or SCONH-,
A^3 is $(CH_2)_r$, $O(CH_2)_r$, $NH(CH_2)_r$, $S(CH_2)_r$ or -(CHQ)-, wherein
r is an integer from 1 to 6 and
Q is a substituted or unsubstituted alkyl or aryl group,
sp is a divalent spacer or a bond, and
k is an integer from 5 to 100.

The preparation of the compounds of the general formula (I) will now be described. The compounds of formula (III) can also be prepared in accordance with this preparation method.

The synthesis of the compounds of the general formula (I) is advantageously carried out in each case starting from the corresponding tetramines by successive chain lengthening (Scheme 1), in which procedure known methods of peptide chemistry are used, the Boc group being used as N-protecting group. The amide bonds are preferably formed using the active ester method.

Scheme 1

$[H_2NCH_2-]_4C \rightarrow [BocNH(CH_2)_pCONHCH_2-]_4C \rightarrow [H_2N(CH_2)_pCONHCH_2-]_4C \rightarrow \rightarrow$
$[H\text{-}AC_mGly_nNHCH_2-]_4C$

p = 1 or 6, n = 0 to 7, m = 0 to 3

The terminal groups are advantageously linked likewise by way of the active ester method to the compounds of the general formula (I) synthesised according to Scheme 1 (Scheme 2).

Scheme 2

$[H\text{-}AC_mGly_nNHCH_2-]_4C$ + Sug-sp-AC_m-Ad-ONp $\rightarrow \rightarrow$ [Sug-sp-AC_m-Ad-$AC_mGly_nNHCH_2-]_4C$

Sug-sp- =	Neu5Acα2-$OCH_2(p\text{-}C_6H_4)NHCOCH_2NH$-	**(Neu5Ac-Gab-)**
	Neu5Acα2-$O(CH_2)_3NH$-	**(Neu5Ac-Ap-)**
	Neu5Acα2-3Galβ1-4Glcβ1-NHCOCH$_2$NH-	**(3`SL-NHCOCH$_2$NH$_2$-)**
	Galα1-3Galβ1-$O(CH_2)_3NH$-	**(B_{dl}-Ap-)**

The formation of aggregates will now be described in detail and with reference to the Figures.

Fig. 1 shows elution profiles of aggregates $\{[Neu5Ac\text{-}Gab\text{-}AC_m\text{-}Ad\text{-}Gly_5\text{-}NHCH_2-]_4C\}_x$, HPLC, TSK-4000, 0.2M NaCl;

Fig. 2 shows the relative particle size distribution of aggregate $\{[Neu5Ac\text{-}Gab\text{-}Ad\text{-}AC_3\text{-}Gly_5\text{-}NHCH_2-]_4C\}_x$, 20°C H_2O;

Fig. 3 shows the influence of temperature and of the presence of urea on the particle size of aggregate {[Neu5Ac-Gab-Ad-Gly_7-$NHCH_2$-]$_4$C }$_x$

The aggregates are high molecular weight non-covalent polymers that are formed by self-association of compounds of the general formula (I) (Scheme 3).

Scheme 3

$$X[A^1\text{-}(A^2\text{-}A^3)_k\text{-}sp\text{-}R]_m \Leftrightarrow \{X[A^1\text{-}(A^2\text{-}A^3)_k\text{-}sp\text{-}R]_m\}_n$$

That intermolecular association takes place spontaneously and results in the formation of stable and ordered structures. The course of that process depends upon the molecular structure of the compounds of the general formula (I) used and upon the external conditions. The molar masses, sizes and shapes of the aggregates formed are likewise determined by those factors.

The non-covalent nature of the bonds between the compounds of the general formula (I) gives rise to the reversibility of the aggregate formation and, in the event of a change in the external conditions, allows dissociation of the aggregates to form compounds of the general formula (I) or conversion thereof into other aggregates, in each case with a view to forming the most thermodynamically stable structures.

The self-association of compounds of the general formula (I) to form aggregates can be observed both in solution and on surfaces.

By means of scanning tunnel microscopy (STM) and atomic force microscopy it has been shown that the aggregate {[Neu5Ac-Gab-Ad-Gly_7-$NHCH_2$-]$_4$C}$_x$ forms ordered chain structures on a graphite substrate.
The formation of aggregates in solution can be observed by light scattering experiments or by gel permeation chromatography.

The compound of the general formula (I) Neu5Ac-Gab-AC_m-Ad-Gly_5-$NHCH_2$-]$_4$C (m=1-3) associates at room temperature in aqueous and organic solvents. Investigation into the associates formed in water using gel permeation chromatography showed the formation of aggregates having molecular weights of about 2000 kD, as shown in Figure 1.

Investigation into the association of the compound of the general formula (I) [Neu5Ac-Gab-Ad-AC_3-Gly_5-$NHCH_2$-]$_4$C in water at 20°C showed the formation of three types of aggregates having particle sizes between 25 and 2000 nm (Figure 2). When the sample was heated to 60°C, a reduction in the relative proportion of the smaller particles was observed, while at the same time the relative proportion of the larger particles increased and the total number of particles decreased. An increase in aggregate size with temperature was also observed in the case of the compound of the general formula (I) **(48)**. That compound forms in water at 60°C particles having sizes of up to 8000 nm (Figure 3).

External conditions that determine the formation of the aggregates and the course of the intermolecular association include, in addition to temperature, the pH value and the nature and composition of the solvent. By means of light scattering experiments it has been shown that the compound [HCl·H-Gly_7-$NHCH_2$-]$_4$C (**22a**) in water at 20°C is present in non-associated form, but by the addition of a 0.8M $NaHCO_3$ solution a self-association of the compound is achieved. The addition of HCl then enables the association to be reversed again (cf. Example 9).

The formation of aggregates is also influenced by the presence of components that are able to enter into interactions with the compounds of the general formula (I). Those components may be organic molecules, such as, for example, compounds of the formula (III), urea (Figure 3), trifluoroethanol, methanol, acetone or other organic solvents. There may also be other compounds of the general formula (I) or (III) that on their own - under the given conditions - do not form associates.

In the case of compounds of the general formula (I) and aggregates, the process of self-association is influenced also by the interactions between the ligands and the corresponding receptors. That influence may, for example, be such that only as a result of the presence of the receptors is an association of compounds of the general formula (I) brought about, more specifically under conditions in which association of those compounds would not otherwise take place. As a result of the reversibility of the aggregate formation it is equally possible that aggregates, in the presence of receptors, change in such a manner, with rearrangement or modification of the composition, that a thermodynamically advantageous state of the entire system consisting of aggregate and receptor is achieved. The aggregates can therefore adapt themselves to different receptor arrangements and thus optimise an interaction between the receptors and

ligands. That optimisation by subsequent adaptation of the polyvalent interactions constitutes a substantial advantage over the prior art.

Special biologically active aggregates will now be described. As a result of the self-association of compounds of the general formula (I) with biologically active ligands there are formed biologically active aggregates that act as highly effective multivalent inhibitors of biological recognition processes. The specific activity of such an inhibitor is dependent upon the affinity of terminal groups R, and also upon the "matrix" of the aggregate, that is to say the structure of the compound of the general formula (I) used as carrier.

Tables 2 and 3 show the influence of the matrix structure on the inhibition of the viral cell adhesion of influenza viruses, measured in a fetuin binding assay known to the person skilled in the art. That assay reveals an increase in the specific activity of the inhibitor by more than three orders of magnitude in comparison with the activity of the free ligand Neu5AcαBn in the case of the aggregate $\{[Neu5Ac\text{-}Gab\text{-}Ad\text{-}AC_3\text{-}Gly_5\text{-}NHCH_2\text{-}]_4C\}_x$ (**44**).

Table 1

Inhibition of viral cell adhesion of influenza viruses, strain A/NIB/44/90M H3N2, FBI test, Neu5AcαBn as a reference compound, specific activity per Neu5Ac group

Inhibitor	Relative activity
Neu5Acα-OBn	1
$[Neu5Ac\text{-}Gab\text{-}Ad\text{-}Gly_n\text{-}NHCH_2\text{-}]_4C$ (n=0-5)	2
$[Neu5Ac\text{-}Ap\text{-}Ad\text{-}Gly_n\text{-}NHCH_2\text{-}]_4C$ (n=3-5)	1
$[Neu5Ac\text{-}Gab\text{-}Ad\text{-}GlyGluGly\text{-}NHCH_2\text{-}]_4C$	5
$[Neu5Ac\text{-}Gab\text{-}AC\text{-}Ad\text{-}Gly_5\text{-}NHCH_2\text{-}]_4C$	15
$[Neu5Ac\text{-}Gab\text{-}AC_2\text{-}Ad\text{-}Gly_5\text{-}NHCH_2\text{-}]_4C$	330
$[Neu5Ac\text{-}Gab\text{-}AC_3\text{-}Ad\text{-}Gly_5\text{-}NHCH_2\text{-}]_4C$	1000
$[Neu5Ac\text{-}Gab\text{-}Ad\text{-}AC_2\text{-}Gly_5\text{-}NHCH_2\text{-}]_4C$	1000
$[Neu5Ac\text{-}Gab\text{-}Ad\text{-}AC_3\text{-}Gly_5\text{-}NHCH_2\text{-}]_4C$	2500

Table 2

Inhibition of viral cell adhesion of influenza viruses inhibition of strain A/Duck/Alberta/60/67 H12N5, FBI test, 3`SL as a reference compound, specific activity per 3`SL group

Inhibitor	Relative activity
3`SL	1
[3`SL-NHCOCH$_2$NH-Ad-Gly$_5$-NHCH$_2$-]$_4$C	20
[3`SL-NHCOCH$_2$NH-Ad-Gly$_7$-NHCH$_2$-]$_4$C	200

A further example of the increase in biological activity of a biological ligand resulting from its binding to an aggregate is the compound $\{[B_{di}\text{-}Ap\text{-}Ad\text{-}AC_3\text{-}Gly_5\text{-}NHCH_2\text{-}]_4C\}_x$ (**49**) as inhibitor of the cytotoxicity of human blood sera with respect to porcine kidney cells PK15. The aggregate (**49**) exhibits a specific activity three orders of magnitude higher than the free ligand Galα1-3Gal (B disaccharide).

Abbreviations used:

Np	para-nitrophenyl
NOS	N-oxysuccinimidyl
Boc	tert-butyloxycarbonyl
AC	6-aminocaproyl
Ad	1,6-hexanedioyl
Ap	3-aminopropyl
Gab	4-(glycylamido)-benzyl
Sug	carbohydrate radical
SL	sialyl lactose
Bn	benzyl
LC	column chromatography
TLC	thin-layer chromatography

The invention will now be described in greater detail with reference to Examples.

Materials and methods:

^{1}H-NMR spectra (δ, ppm, TMS) were recorded using a spectrometer of the WM-500 type from Bruker (USA) at 303°K.

Mass spectra were recorded using a time-of-flight spectrometer of the MSBCh type (Sumy, Ukraine) (ionisation by cleavage products of californium-252 at an acceleration voltage of +15 eV).

The light scattering experiments were carried out using the following apparatus: Coultronics Coulter N4-MD (He-Ne laser, λ=632.8 nm, measurement of the scattering at an angle of 62.5° to the incident light beam), Spectra-Physics 164 (argon laser, λ=528.7 nm and λ=611.5 nm, measurement of the scattering at an angle of 90° to the incident light beam).

Silica gel 60 (40-63 µm) (Merck) was used for column chromatography. Sephadex of types LH-20, G-10, G-25 (Pharmacia, Sweden) and TSK-4000 (HPLC) were used for gel permeation chromatography.

For TLC, silica gel 60 (Merck) and silica gel 60 glass plates with fluorescent indicator F254 (Merck) were used. For the detection of spots on the TLC plates, the following methods were used:

- heating after spraying with a 7% H_3PO_4 solution (carbohydrate compounds);
- heating after spraying with a 2% ninhydrin solution in ethanol (compounds having primary amino groups);
- heating after a dwell time of 10 minutes in a chamber over conc. HCl and subsequent spraying with a 2% ninhydrin solution in ethanol (compounds having Boc-protected amino groups);
- dwell time of 10 minutes in a chamber over conc. NH_3 (4-nitrophenyl ester);
- observing the plates under UV.

For TLC, the following eluant systems were used:

A – toluene/ethyl acetate 2:1

B – acetone/ethyl acetate/methanol 10:4:1

C – $CHCl_3$/MeOH 7:1

D – $CHCl_3$/ethyl acetate/MeOH/AcOH 9:3:2:0.2

E – iPrOH/ethyl acetate/H_2O 2:3:1

F – EtOH/$NH_{3\,(aq)}$ 2:1

G – iPrOH/ethyl acetate/H_2O 4:3:2

H – iPrOH/acetone/H_2O 4:3:2

Preparation of known starting compounds

Tetrakis(aminomethyl)methane tetrahydrochloride **(1)**
was prepared analogously to the literature (E. B. Fleischer, A.E. Gebala, A. Levey, P.A. Tasker, *J.Org.Chem.*, **36**, 3042, 1971).
TLC: R_f=0.6; eluant – 25% ammonia/water; developer – ninhydrin.
M.p. >300°C.
^{1}H-NMR spectrum in D_2O (δ, ppm): 3.45 (s, CH_2).

4-Nitrophenyl trifluoroacetate **(2)**
was prepared analogously to the literature (S. Sakakibara, N. Inukai, *Bull.Chem.Soc.Jap.*, **37**, 1231, 1964).

Di-(4-nitrophenyl) adipate **(3)**
was prepared analogously to the literature (S. Sakakibara, N. Inukai, *Bull.Chem.Soc.Jap.*, **37**, 1231, 1964).
R_f=0.76, eluant Ð A.
^{1}H-NMR spectrum in $CDCl_3$ (δ, ppm): 1.871 (m, 4H, 2 $COCH_2C\underline{H}_2$), 2.666 (m, 4H, 2 $COCH_2$), 7.255 and 8.240 (m, 8H, $J_{2,3}$ 9Hz, Ar).

Methyl [4-(tert-butyloxycarbonyl-glycilamido)benzyl 5-acetamido-4,7,8,8-tetra-O-acetyl-3,5-didesoxy-α-D-glycero-D-galacto-nonulopyranosid]oate
Ac_4(OMe)Neu5Ac-Gab-Boc (4)
was prepared analogously to the literature (US Patent 5,571,836, 1996).
^{1}H-NMR spectrum ($CDCl_3$, δ, ppm):1.448 (s, 9H, CMe_3), 1.849, 1.994, 2.008, 2.111, 2.127 (s, 5x3H, 5 Ac), 1.979 (dd, 1H, H-3_{ax} Neu5Ac), 2.613 (dd, 1H, J_4 4.6 Hz, J_{3ax} 12.9 Hz, H-3_{eq} Neu5Ac), 3.637 (s, 3H, $COOCH_3$), 3.882 (d, 2H, J 6 Hz, $COC\underline{H}_2NH$), 4.058 (ddd, 1H, H-5 Neu5Ac), 4.074 (dd, 1H, J_{9b} 12.5 Hz, J_8 5.9 Hz, H-9a Neu5Ac), 4.112 (dd, 1H, J_5 10.6, J_7 2.3 Hz, H-6 Neu5Ac), 4.299 (dd, 1H, J_{9b} 12.5 Hz, J_8 2.7 Hz, H-9b Neu5Ac), 4.366 and 4.735 (d, 2x1H, J 12 Hz, $OC\underline{H}_2Ar$), 4.847 (ddd, 1H, J_5 10 Hz, J_{3ax} 12.3 Hz, J_{3eq} 4.6 Hz, H-4 Neu5Ac), 5.24 (br., 1H, N<u>H</u>Boc), 5.251 (d, 1H, J_5 9.8 Hz, NH), 5.314 (dd, 1H, J_6 2.3 Hz, J_8 8.2 Hz, H-7 Neu5Ac), 5.424 (ddd, 1H, H-8 Neu5Ac), 7.258 and 7.445 (d, 2x2H, J 8.4 Hz, Ar), 8.144 (br. s, 1H, N<u>H</u>Ar).

Neu5Acα2-3Galβ1-4Glcβ-NHCOCH$_2$NH$_2$ (12)
was prepared analogously to the literature (L.M. Likhosherstov, O.S. Novikova, V.A. Derevitskaja, N.K. Kochetkov, *Carbohydrate Research*, **146**, C1-C5, 1986; and I.D. Manger, T.W. Rademacher, R.A. Dwek, *Biochemistry*, **31**, 10724, 1992).
^{1}H-NMR spectrum (D_2O, δ, ppm): 1.82 (dd, 1H, H-3$_{ax}$ Neu5Ac, J_4 12 Hz), 2.06 (s, 3H, NAc), 2.79 (dd, 1H, H-3$_{eq}$ Neu5Ac, J_{3ax} 12.4 Hz, J_4 4.6 Hz), 3.48 (m, 1H, H-2 Glc, J_3 9 Hz), 3.61 (dd, 1H, H-2 Gal), 3.99 (dd, 1H, H-4 Gal), 4.14 (dd, 1H, H-3 Gal, J_2 9.8 Hz, J_4 3.1 Hz), 4.57 (d, 1H, H-1 Gal, J_2 7.8 Hz), 5.09 (d, 1H, H-1 Glc, J_2 9.3 Hz).

Galα1-3Galβ-O(CH$_2$)$_3$NH$_2$ (13)
was prepared analogously to the literature (E. Yu. Korchagina, N. V. Bovin, *Bioorganicheskaya Khimiya*, 1992, **18**, 283, Rus).

The compounds *BocGlyNOS*, *BocGlyGlyNOS* and *BocAC-ONp* were prepared using N,N'-dicyclohexylcarbodiimide analogously to the literature (G. W. Anderson, J. E. Zimmerman, F. M. Callahan, *J. Amer. Chem. Soc.*, **86**, 1839, 1964; M. Bodanszky, V. du Vigneaud, *J. Amer. Chem. Soc.*, **81**, 5688, 1959).

Example 1.

Preparation of **Ac$_4$(OMe)Neu5Ac-Gab-AC-Boc (5)**.
10 ml of $CHCl_3$ and 2 ml of CF_3COOH were added to 0.5 mmol of compound **(4)**. The reaction mixture was stirred at room temperature for one hour; 2 ml of toluene were added and the mixture was concentrated by evaporation *in vacuo* and dried. The residue was dissolved in 10 ml of $CHCl_3$, and 1.5 mmol of 6-N-Boc-amino-(4-nitrophenyl) hexanoate and 0.3 ml of NEt_3 were added. The reaction mixture was stirred at room temperature for 24 hours and concentrated by evaporation *in vacuo*. The resulting residue was chromatographed over silica gel.

The compounds **Ac$_4$(OMe)Neu5Ac-Gab-AC$_2$-Boc (6)** and **Ac$_4$(OMe)Neu5Ac-Gab-AC$_3$-Boc (7)** were prepared in an analogous manner (see Table 4).

Table 3 (Example 1)

Product	Starting compound	TLC: eluant A, R_f	Column chromatography	Yield, %
Ac_4(OMe)Neu5Ac-Gab-AC-Boc (5)	(4)	0.6	$CHCl_3$/MeOH 35:1 → 10:1	90
Ac_4(OMe)Neu5Ac-Gab-AC_2-Boc (6)	(5)	0.45	acetone/ethyl acetate/MeOH 10:4:0.5 → 10:4:3	72
Ac_4(OMe)Neu5Ac-Gab-AC_3-Boc (7)	(6)	0.25	acetone/ethyl acetate/MeOH 10:4:1 → 10:4:5	70

^{1}H-NMR spectra ($CDCl_3$, δ, ppm):

Ac_4(OMe)Neu5Ac-Gab-AC-Boc (5): 1.331, 1.468, 1.655 (m, $3CH_2$), 1.402 (s, 9H, CMe_3), 2.264 (t, 2H, J 7.5 Hz, $C\underline{H}_2CONHCH_2CO$), 3.066 (m ~ quadr, 2H, J 6.6 Hz, $C\underline{H}_2NHBoc$), 4.060 (d, 2H, J 5 Hz, $COC\underline{H}_2NH$), 4.364 and 4.733 (d, 2x1H, J 12 Hz, $OC\underline{H}_2Ar$), 4.571 (br., 1H, N<u>H</u>Boc), 6.521 (br., 1H, $COCH_2N\underline{H}CO$), 7.253 and 7.460 (d, 2x2H, J 8.4 Hz, Ar), 8.547 (br. s, 1H, N<u>H</u>Ar).
Neu5Acα fragment: (see **(4)**).

Ac_4(OMe)Neu5Ac-Gab-AC_2-Boc (6): 1.280, 1.338, 1.447, 1.482, 1.582, 1.655, 2.107 (m, $7CH_2$), 1.403 (s, 9H, CMe_3), 2.276 (t, 2H, J 7.2 Hz, $C\underline{H}_2CONHCH_2CO$), 3.060 (m ~ quadr, 2H, J 6.6 Hz, $C\underline{H}_2NHBoc$), 3.216 (m ~ quadr, 2H, J 6.4 Hz, $C\underline{H}_2NH$), 4.040 (d, 2H, J 5 Hz, $COC\underline{H}_2NH$), 4.353 and 4.728 (d, 2x1H, J 12 Hz, $OC\underline{H}_2Ar$), 4.651 (br., 1H, N<u>H</u>Boc), 5.793 (t, 1H, J 5 Hz, $CH_2N\underline{H}CO$), 6.714 (br., 1H, $COCH_2N\underline{H}CO$), 7.245 and 7.467 (d, 2x2H, J 8.4 Hz, Ar), 8.666 (br. s, 1H, N<u>H</u>Ar). Neu5Acα fragment: (see **(4)**).

Ac_4(OMe)Neu5Ac-Gab-AC_3-Boc (7): 1.283, 1.336, 1.447, 1.482, 1.594, 1.655, 2.117 (m, $11CH_2$), 1.401 (s, 9H, CMe_3), 2.282 (t, 2H, J 7.2 Hz, $C\underline{H}_2CONHCH_2CO$), 3.045 (m ~ quadr, 2H, J 6.6 Hz, $C\underline{H}_2NHBoc$), 3.214 (m ~ quadr, 4H, J 6.4 Hz, $C\underline{H}_2NH$), 4.040 (d, 2H, J 5 Hz, $COC\underline{H}_2NH$), 4.353 and 4.728 (d, 2x1H, J 12 Hz, $OC\underline{H}_2Ar$), 4.669 (br., 1H, N<u>H</u>Boc), 5.876 (t, 1H, J 5.5 Hz, $CH_2N\underline{H}CO$), 6.071 (br., 1H, $CH_2N\underline{H}CO$), 6.940 (br., 1H, $COCH_2N\underline{H}CO$), 7.242 and 7.483 (d, 2x2H, J 8.4 Hz, Ar), 9.033 (br. s, 1H, N<u>H</u>Ar).
Neu5Acα fragment: (see (4)).

<u>Example 2.</u>

Preparation of **Ac_4(OMe)Neu5Ac-Gab-AC-Ad-ONp (9).**

10 ml of $CHCl_3$ and 2 ml of CF_3COOH were added to 0.5 mmol of compound **(5)**. The reaction mixture was stirred at room temperature for one hour; 5 ml of toluene were added and the mixture was concentrated by evaporation *in vacuo* and dried. The residue

was dissolved in 15 ml of tetrahydrofuran; 5 mmol of compound **(3)** and 0.3 ml of NEt_3 were added and the reaction mixture was stirred at room temperature for 24 hours. The excess NEt_3 was neutralised with CH_3COOH and the reaction mixture was concentrated by evaporation. The residue was dissolved in $CHCl_3$ and the resulting solution was washed with water and concentrated by evaporation. The resulting mixture was chromatographed over a column of silica gel (see Table 4).

The compounds **Ac_4(OMe)Neu5Ac-Gab-Ad-ONp (8)**, **Ac_4(OMe)Neu5Ac-Gab-AC_2-Ad-ONp (10)** and **Ac_4(OMe)Neu5Ac-Gab-AC_3-Ad-ONp (11)** were prepared in an analogous manner (see Table 4).

Table 4 (Example 2)

Product	Starting compound	TLC: eluant C, R_f	Column chromatography	Yield, %
Ac_4(OMe)Neu5Ac-Gab-Ad-ONp (8)	(4)	0.6	$CHCl_3$/*i*-PrOH 20:1	78
Ac_4(OMe)Neu5Ac-Gab-AC-Ad-ONp (9)	(5)	0.55	$CHCl_3$/MeOH/AcOH 35:1:0.2 → 15:1:0.2	65
Ac_4(OMe)Neu5Ac-Gab-AC_2-Ad-ONp (10)	(6)	0.48	$CHCl_3$/MeOH/AcOH 35:1:0.2 → 15:1:0.2	60
Ac_4(OMe)Neu5Ac-Gab-AC_3-Ad-ONp (11)	(7)	0.43	$CHCl_3$/MeOH/AcOH 35:1:0.2 → 15:1:0.2	62

^{1}H-NMR-spectra:

Ac_4(OMe)Neu5Ac-Gab-Ad-ONp (8) ($CDCl_3$, δ, ppm): 1.774 (m, 2H, $C\underline{H}_2CH_2COO$), 1.843, 1.984, 2.00, 2.100, 2.117 (s, 5x3H, 5 Ac), 1.966 (dd, 1H, H-3_{ax} Neu5Ac), 2.335 and 2.393 (m, 2x1H, $C\underline{H}_2CH_2CONH$), 2.601 (t, 2H, J 6Hz, $CH_2C\underline{H}_2COO$), 2.604 (dd, 1H, H-3_{eq} Neu5Ac), 3.645 (s, 3H, $COOCH_3$), 3.688 (t, 2H, J 4.7Hz, $CH_2C\underline{H}_2CONH$), 4.049 (ddd, 1H, H-5 Neu5Ac), 4.062 (dd, 1H, J_8 6_Hz, H-9a Neu5Ac), 4.074 (d, 2H, J_{NH} 5.5Hz, $COC\underline{H}_2NHCO$), 4.111 (dd, 1H, J_5 10.7, J_7 2.3Hz, H-6 Neu5Ac), 4.298 (dd, 1H, J_{9b} 12.5Hz, J_8 2.9Hz, H-9b Neu5Ac), 4.343 and 4.722 (d, 2x1H, J 12Hz, $OC\underline{H}_2Ar$), 4.839 (ddd, 1H, J_5 10.2Hz, J_{3ax} 12.3Hz, J_{3eq} 4.6Hz, H-4 Neu5Ac), 5.307 (dd, 1H, J_8 8.4Hz, J_6 2.3Hz, H-7 Neu5Ac), 5.359 (d, 1H, J_5 9.7Hz, NH), 5.406 (ddd, 1H, H-8 Neu5Ac), 6.616 (t, 1H, $COCH_2N\underline{H}CO$), 7.243 and 7.450 (d, 2x2H, J 8.5Hz, *p*-$C_6\underline{H}_4NH$), 7.221 and 8.208 (d, 2x2H, J 9Hz, *p*-$C_6\underline{H}_4NO_2$), 8.586 (s, 1H, $N\underline{H}Ar$).

Ac_4(OMe)Neu5Ac-Gab-AC-Ad-ONp (9) ($CDCl_3$, δ, ppm): 1,341 (m, 2H, $COCH_2CH_2C\underline{H}_2CH_2CH_2NH$), 1.495 and 1.666 (m, 2x2H, $COCH_2C\underline{H}_2CH_2C\underline{H}_2CH_2NH$), 1.729 (m, 2H, $\underline{CH}_2CH_2COO$), 1.856, 1.991, 2.010, 2.110 and 2.129 (s, 5x3H, 5 Ac), 1.976 (dd, 1H, H-3_{ax} Neu5Ac), 2.138, 2.175 (m, 2x1H, $C\underline{H}_2CH_2CONH$), 2.182 and 2.267 (t,

2x2H, 2 CH_2CONH), 2.601 (~t, 2H, J 6.8Hz, CH_2CH_2COO), 2.611 (dd, 1H, J_{3ax} 12.8, J_4 4.5Hz, H-3_{eq} Neu5Ac), 3.228 (m ~ quadr, 2H, J 6.6Hz, CH_2NHCO), 3.645 (s, 3H, $COOCH_3$), 4.022 (d, 2H, J_{NH} 5.4Hz, $COCH_2NHCO$), 4.050 (ddd, 1H, H-5 Neu5Ac), 4.065 (dd, 1H, J_8 6Hz, H-9a Neu5Ac), 4.113 (dd, 1H, J_5 10.8, J_7 2.3Hz, H-6 Neu5Ac), 4.295 (dd, 1H, J_{9a} 12.5Hz, J_8 2.9Hz, H-9b Neu5Ac), 4.357 and 4.732 (d, 2x1H, J 12Hz, OCH_2Ar), 4.848 (ddd, 1H, J_5 10Hz, J_{3ax} 12.2Hz, J_{3eq} 4.5Hz, H-4 Neu5Ac), 5.170 (d, 1H, J_5 10Hz, NH), 5.308 (dd, 1H, J_8 8.6Hz, J_6 2.3Hz, H-7 Neu5Ac), 5.413 (ddd, 1H, H-8 Neu5Ac), 5.708 (t, 1H, CH_2CH_2NHCO), 6.483 (t, 1H, $COCH_2NHCO$), 7.251 and 7.427 (d, 2x2H, J 8.7Hz, *p*-C_6H_4NH), 7.243 and 8.224 (d, 2x2H, J 9Hz, *p*-$C_6H_4NO_2$), 8.298 (s, 1H, NHAr).

Ac_4(OMe)Neu5Ac-Gab-AC_2-Ad-ONp (10) (D_6-DMSO, δ, ppm): 1.231, 1.376, 1.485 and 1.608 (m, CH_2), 1.757 (dd, 1H, H-3_{ax} Neu5Ac), 1.678, 1.917, 1.974, 2.023 and 2.092 (s, 5x3H, 5 Ac), 2.570 (dd, 1H, J_{3ax} 12.4, J_4 4.5Hz, H-3_{eq} Neu5Ac), 2.638 (t, 2H, J 7Hz, CH_2CH_2COO), 3.009 (m, 4H, $2CH_2NHCO$), 3.699 (s, 3H, $COOCH_3$), 3.859 (d, 2H, J_{NH} 5.9Hz, $COCH_2NHCO$), 3.904 (ddd, 1H, H-5 Neu5Ac), 4.027 (dd, 1H, J_8 6.2Hz, H-9a Neu5Ac), 4.089 (dd, 1H, J_5 10.8, J_7 2.6Hz, H-6 Neu5Ac), 4.235 (dd, 1H, J_{9a} 12.4Hz, J_8 3.1Hz, H-9b Neu5Ac), 4.322 and 4.645 (d, 2x1H, J 11.7Hz, OCH_2Ar), 4.715 (ddd, 1H, J_5 10Hz, J_{3ax} 12Hz, J_{3eq} 4.5Hz, H-4 Neu5Ac), 5.193 (dd, 1H, J_8 8.4Hz, J_6 2.Hz, H-7 Neu5Ac), 5.341 (ddd, 1H, H-8 Neu5Ac), 7.216 and 7.554 (d, 2x2H, J 8.4Hz, *p*-C_6H_4NH), 7.433 and 8.296 (d, 2x2H, J 9.2Hz, *p*-$C_6H_4NO_2$), 7.674 (t, 1H, J 5.5Hz, CH_2CH_2NHCO), 7.706 (d, 1H, J_5 9.8Hz, NH), 7.754 (t, 1H, J 5.8Hz, CH_2CH_2NHCO), 8.081 (t, 1H, J 5.9Hz, $COCH_2NHCO$), 9.961 (s, 1H, NHAr).

Ac_4(OMe)Neu5Ac-Gab-AC_3-Ad-ONp (11) (D_6-DMSO, δ, ppm): 1.214, 1.360, 1.478, 1.609 (m, CH_2), 2.639 (t, 2H, J 7Hz, CH_2CH_2COO), 2.999 (m, 6H, $3CH_2NHCO$), 3.864 (d, 2H, J_{NH} 5.9Hz, $COCH_2NHCO$), 4.324 and 4.645 (d, 2x1H, J 11.7Hz, OCH_2Ar), 7.212 and 7.568 (d, 2x2H, J 8.4Hz, *p*-C_6H_4NH), 7.435 and 8.295 (d, 2x2H, J 9.2Hz, *p*-$C_6H_4NO_2$), 7.700 (m, 2H, $2CH_2CH_2NHCO$), 7.750 (t, 1H, J 5.8Hz, CH_2CH_2NHCO), 8.122 (t, 1H, J 5.9Hz, $COCH_2NHCO$), 10.047 (s, 1H, NHAr), Neu5Acα fragment: see **(10)**.

Example 3.

Preparation of

Neu5Acα2-3Galβ1-4Glcβ-$NHCOCH_2NHCO(CH_2)_4COO$(4-$C_6H_4NO_2$) (14).

119 mg (0.172 mmol) of compound (**12**) in 0.5 ml of DMSO were added, while stirring, to a solution of 334 mg (0.86 mmol) of compound (**3**) in 2 ml of DMF. The mixture was stirred at 20°C for 24 hours. After the addition of 200 µl of AcOH, the reaction mixture was diluted with 15 ml of water. The solution was filtered and the filtrate was concentrated

to a volume of ~ 2 ml. The residue was poured onto a Sephadex LH-20 column (1.5x50 cm) and eluted with MeCN/H_2O (1:1, 0.2% AcOH). After isolation, 140 mg of (**14**) were obtained, corresponding to a yield of 87 %. TLC: R_f 0.41 (eluant H).

^{1}H-NMR spectrum (D_2O, δ, ppm): 1.737 (m, 1H, $C\underline{H_2}C\underline{H_2}CH_2CO$), 1.779 (dd, 1H, H-$3_{ax}$ Neu5Ac, J_4 12.5 Hz), 2.003 (s, 3H, NAc), 2.383 (t, 1H, J 7 Hz, CH_2CO), 2.733 (dd, 1H, H-3_{eq} Neu5Ac, J_{3ax} 12.5 Hz, J_4 4.5 Hz), 3.432 (m, 1H, H-2 Glc, J_3 9 Hz), 3.556 (dd, 1H, H-2 Gal), 3.933 (dd, 1H, H-4 Gal), 4.090 (dd, 1H, H-3 Gal, J_2 10 Hz, J_4 3 Hz), 4.499 (d, 1H, H-1 Gal, J_2 8 Hz), 4.985 (d, 1H, H-1 Glc, J_2 9 Hz).

The compound **Neu5Ac-Gab-Ad-ONp** (**15**) was prepared analogously starting from (**3**) and Neu5Acα-OCH_2(p-C_6H_4)-$NHCOCH_2NH_2$ (US Patent 5,571,836, 1996).
^{1}H-NMR spectrum (CD_3OD, δ, ppm): 1.968 (dd, 1H, H-3_{ax} Neu5Ac), 1.980 (m, 4H, $C\underline{H_2}C\underline{H_2}CH_2CO$), 2.205 (s, 3H, $NCOCH_3$), 2.565 and 2.874 (t, 2x2H, J 6.8 Hz, 2 CH_2CO), 2.976 (dd, 1H, J_4 4.5 Hz, J_{3ax} 13 Hz, H-3_{eq} Neu5Ac), 3.743 (dd, 1H, J_6 1.5 Hz, J_8 9 Hz, H-7 Neu5Ac), 3.821 (dd, 1H, J_5 10 Hz, H-6 Neu5Ac), 3.840 (dd, 1H, J_{9b} 12 Hz, J_8 6 Hz, H-9a Neu5Ac), 3.924 (ddd, 1H, H-4 Neu5Ac), 3.978 (ddd, 1H, H-5 Neu5Ac), 4.047 (dd, 1H, J_8 2 Hz, H-9b Neu5Ac), 4.083 (ddd, 1H, H-8 Neu5Ac), 4.196 (s, 2H, $COC\underline{H_2}NH$), 4.653 and 4.973 (d, 2x1H, J 11 Hz, $OC\underline{H_2}Ar$), 7.474 and 7.707 (d, 2x2H, J 8.3 Hz, p-$C_6\underline{H_4}NH$), 7.561 and 8.467 (d, 2x2H, J 8.8 Hz, p-$C_6\underline{H_4}NO_2$).

The compound **Galα1-3Galβ-O$(CH_2)_3NHCO(CH_2)_4COO(p$-$C_6H_4NO_2)$ (16)** was prepared analogously starting from (**3**) and Galα1-3Galβ-$O(CH_2)_3NH_2$ (**13**).
^{1}H-NMR spectrum (D_2O, δ, ppm): 1.78 (m, 4H, CH_2CH_2), 1.89 (m, 2H, CH_2), 2.36 (t, 2H, CH_2COO), 2.77 (m, 2H, $NHCOC\underline{H_2}$), 3.36 (m, 2H, CH_2N), 3.69 (t, 1H, J_3 9 Hz, 2-Galβ), 3.76 (m, 1H, OCH`), 3.78 (m, 6,6`-Galα), 3.91 (dd, 1H, J_3 10 Hz, 2-Galα), 4.00 (dd, 1H, 3-Galα), 4.01 (m, 1H, OCH), 4.06 (br. d, 1H, 4-Galα), 4.20 (br. d, 1H, 4-Galβ), 4.23 (br. t, 1H, 5-Galα), 4.48 (d, 1H, J_2 8 Hz, 1-Galβ), 5.19 (d, 1H, J_2 4 Hz, 1-Galα), 8.38, 7.43 (d, 2x2H, J 9.5 Hz, Ar).

Example 4.

Tetra-(N-tert-butyloxycarbonyl-pentaglycilamidomethyl)methane
[$BocGly_5$-$NHCH_2$-]$_4$C (21).
1 mmol of compound (**19**) (see Table 5) was taken up in 4 ml of CF_3COOH and stirred at room temperature for two hours. 4 ml of toluene were added and the reaction mixture

was concentrated by evaporation *in vacuo* and dried. The residue was dissolved in 5 ml of water; 4 ml of a 2M HCl solution were added and concentration was carried out. The resulting tetrahydrochloride (**19a**) was dried *in vacuo* and suspended in 0.5 ml of DMF; 6 mmol of BocGlyGlyNOS and 0.5 ml of NEt_3 were added and the mixture was stirred at room temperature for 24 hours. The reaction mixture was concentrated *in vacuo* and the product was purified by column chromatography. After drying *in vacuo*, compound (**21**) was obtained in the form of a white powder in a yield of 69% (see Table 5).

Compounds (**17**)-(**20**), (**22**)-(**25**) were prepared in an analogous manner (see Table 5).

^{1}H-NMR spectra (for the allocation of the ^{1}H-NMR signals, the glycines within the chains were numbered, the numbering beginning in each case at the N-terminal end of the chains).

[BocGly-NHCH$_2$-]$_4$C (17). ^{1}H-NMR spectrum in D_6-DMSO (δ, ppm): 1.366 (s, 9H, $OCMe_3$), 2.759 (br. d, 2H, CCH_2), 3.494 (d, 2H, J_{NH} 6 Hz, CH_2^{Gly}), 7.368 (t, 1H, NH^{Gly}), 7.969 (br. t, 1H, $CCH_2N\underline{H}$), mass spectrum: 783 (M+Na).
[HCl·H-Gly$_2$-NHCH$_2$-]$_4$C (18a). ^{1}H-NMR spectrum in D_2O (δ, ppm): 2.952 (s, 2H, CCH_2), 3.966 (s, 2H, CH_2^{Gly}), 4.013 (s, 2H, CH_2^{Gly}).
[BocGly$_3$-NHCH$_2$-]$_4$C (19). ^{1}H-NMR spectrum in D_6-DMSO (δ, ppm): 1.375 (s, 9H, $OCMe_3$), 2.690 (br. d, 2H, J_{NH} 6.5 Hz, CCH_2), 3.586 (d, 2H, J_{NH} 6 Hz, CH_2^{Gly3}), 3.725 (d, 2H, J_{NH} 5.5 Hz, CH_2^{Gly1}), 3.847 (d, 2H, J_{NH} 5.5 Hz, CH_2^{Gly2}), 6.976 (t, 1H, NH^{Gly3}), 7.811 (t, 1H, NH^{Gly2}), 7.975 (t, 1H, $CCH_2\underline{NH}$), 8.534 (t, 1H, NH^{Gly1}), mass spectrum: 1239 (M+Na).
[BocGly$_4$-NHCH$_2$-]$_4$C (20). ^{1}H-NMR spectrum in D_6-DMSO (δ, ppm): 1.374 (s, 9H, $OCMe_3$), 2.694 (br. d, 2H, CCH_2), 3.575 (d, 2H, CH_2^{Gly4}), 3.707 (d, 2H, CH_2^{Gly1}), 3.750 (d, 2H, CH_2^{Gly3}), 3.835 (d, 2H, CH_2^{Gly2}), 6.980 (t, 1H, NH^{Gly4}), 7.827 (t, 1H, $CCH_2\underline{NH}$), 8.048 (t, 1H, NH^{Gly3}), 8.096 (t, 1H, NH^{Gly2}), 8.590 (t, 1H, NH^{Gly1}), mass spectrum: 1467 (M+Na).
[BocGly$_5$-NHCH$_2$-]$_4$C (21). ^{1}H-NMR spectrum in D_6-DMSO (δ, ppm): 1.380 (s, 9H, $OCMe_3$), 2.688 (br. d, 2H, CCH_2), 3.579 (d, 2H, J_{NH} 6 Hz, CH_2^{Gly5}), 3.718 (d, 2H, J_{NH} 5 Hz, CH_2^{Gly1}), 3.750 (d, 4H, J_{NH}~5 Hz, $CH_2^{Gly3,4}$), 3.840 (d, 2H, J_{NH} 5.5 Hz, CH_2^{Gly2}), 6.974 (t, 1H, NH^{Gly5}), 7.770 (t, 1H, $CCH_2\underline{NH}$), 8.006 (t, 1H, NH^{Gly4}), 8.075 and 8.102 (t, 1H, $NH^{Gly2,3}$), 8.550 (t, 1H, NH^{Gly1}), mass spectrum: 1695 (M+Na), 1711(M+K).
[BocGly$_7$-NHCH$_2$-]$_4$C (22). ^{1}H-NMR spectrum in D_6-DMSO (δ, ppm): 1.378 (s, 9H, $OCMe_3$), 2.688 (br., 2H, CCH_2), 3.581 (d, 2H, CH_2^{Gly7}), 3.723 (br. d, 2H, CH_2^{Gly1}), 3.751 (m, 8H, CH_2^{Gly3-6}), 3.840 (br. d, 2H, CH_2^{Gly2}), 6.970 (br. t, 1H, NH^{Gly7}), 7.814 (br. t, 1H,

$CCH_2\underline{NH}$), 8.018 (br. t, 1H, NH^{Gly6}), 8.081, 8.085, 8.092 and 8.118 (m, 4H, NH^{Gly2-5}), 8.545 (br. t, 1H, NH^{Gly1}).

[HCl·H-AC-Gly$_5$-NHCH$_2$-]$_4$C (23a). ^{1}H-NMR spectrum in D_2O (δ, ppm): 1.446 (m, 2H, CH_2), 1.689 (m, 2H, $COCH_2C\underline{H_2}$), 1.724 (m, 2H, $C\underline{H_2}CH_2N$), 2.398 (t, 2H, J 7.4 Hz, $COCH_2$), 2.967 (br. s, CCH_2), 3.044 (t, 2H, J 7.4 Hz, CH_2N), 3.994, 4.012, 4.049 (x2) and 4.096 (s, 10H, 5 $COCH_2N$).

[HCl·H-AC$_2$-Gly$_5$-NHCH$_2$-]$_4$C (24a). ^{1}H-NMR spectrum in D_2O (δ, ppm): 1.336 and 1.382 (m, 4H, 2 CH_2), 1.548 (m, 2H, $C\underline{H_2}CH_2N$), 1.656 (m, 4H, 2 $COCH_2C\underline{H_2}$), 1.712 (m, 2H, $C\underline{H_2}CH_2N^+$), 2.283 (t, 2H, J 7.4 Hz, $COCH_2$), 2.370 (t, 2H, J 7.4 Hz, $COCH_2NHCOC\underline{H_2}$), 2.955 (br. s, CCH_2), 3.031 (t, 2H, J 7.4 Hz, CH_2N^+), 3.206 (t, 2H, J 6.6 Hz, CH_2N), 3.988, 4.00, 4.044 (x2) and 4.091 (s, 10H, 5 $COCH_2N$).

[HCl·H-AC$_3$-Gly$_5$-NHCH$_2$-]$_4$C (25a). ^{1}H-NMR spectrum in D_2O (δ, ppm): 1.34-1.42 (m, 6H, 3 CH_2), 1.551 (m, 4H, 2 $C\underline{H_2}CH_2N$), 1.653 (x2) and 1.689 (m, 6H, 3 $COCH_2C\underline{H_2}$), 1.717 (m, 2H, $C\underline{H_2}CH_2N^+$), 2.270 and 2.288 (t, 4H, J 7.5 Hz, 2 $COCH_2$), 2.376 (t, 2H, J 7.5 Hz, $COCH_2NHCOC\underline{H_2}$), 2.952 (br. s, CCH_2), 3.033 (t, 2H, J 7.5 Hz, CH_2N^+), 3.208 (t, 4H, J 7 Hz, 2 CH_2N), 3.990, 4.004, 4.049 (x2) and 4.097 (s, 10H, 5 $COCH_2N$).

Table 5. Preparation of tetravalent matrices (**17**)-(**25**) (Example 4)

Table 5. Preparation of tetravalent matrices (**17**)-(**25**) (Example 4)

End product	Starting compound	Carboxylate	Conditions	TLC				Isolation of the products	Yield %
				Boc derivative		Tetrahydrochloride			
				Rf	Eluant	Rf	Eluant		
[BocGly-NHCH$_2$-I4C (**17**)	(1)	6 mM BocGlyNOS	24 hours stirring in DMF at room temperature	0.5	D	0.72	F	The reaction mixture was concentrated by evaporation *in vacuo* and (**17**) obtained by crystallisation from trifluoromethanol after addition of $CHCl_3$/ethyl acetate/MeOH (9/3/1).	78
[BocGly$_2$-NHCH$_2$-I4C (**18**)	(1)	6mM BocGlyGlyNOS		-	-	0.72		The product (**18**) is only very slightly soluble, it was isolated by filtration and, after removal of the Boc protecting groups, identified as tetrahydrochloride (**18a**)	95
[BocGly$_3$-NHCH$_2$-I4C (**19**)	(17)			0.79	acetone/ MeOH/H_2O 15:1:1	0.61		The reaction mixture was concentrated by evaporation *in vacuo* Column chromatography: acetone/MeOH/H_2O 30:1:1→15:1:1.	77
[BocGly$_4$-NHCH$_2$-I4C (**20**)	(18)			0.48	E	0.55		The reaction mixture was concentrated by evaporation *in vacuo*. Column chromatography: acetone/MeOH/H_2O 30:1:1→10:1:1	63
[BocGly$_5$-NHCH$_2$-I4C (**21**)	(19)			0.36		0.33			69
[BocGly$_7$-NHCH$_2$-I4C (**22**)	(21)			-	-	0.67	2N HCl	The product (**22**) is only very slightly soluble, it was isolated by filtration and, after removal of the Boc protecting groups, identified as tetrahydrochloride (**22a**)	93
[Boc-AC-Gly$_5$-NHCH$_2$-I4C (**23**)	(21)	12 mM BocNH(CH$_2$)5-COONp	DMSO, 70°C 72 hours	0.38	G	0.22		The products, after chromatography over Sephadex LH-20, MeCN/H_2O 1:1 and after removal of the Boc protecting groups, identified as tetrahydrochlorides (**23a**), (**24a**) and (**25a**)	71
[Boc-AC$_2$-Gly$_5$-NHCH$_2$-I4C (**24**)	(23)			0.73		0.1			57
[Boc-AC$_3$-Gly$_5$-NHCH$_2$-I4C (**25**)	(24)			0.4		-			40

Example 5.

Preparation of protected tetrasialosides

Preparation of *[Ac_4(OMe)Neu5Acα-OCH_2(p-C_6H_4)$NHCOCH_2NH$-$CO(CH_2)_4CO$-$(NHCH_2CO)_5NHCH_2]_4C$*

[Ac_4(OMe)Neu5Ac-Gab-Ad-Gly_5-$NHCH_2$-]$_4$C (31).

1 mmol of compound (**21**) (see Table 6) was taken up in 4 ml of CF_3COOH and the mixture was stirred at room temperature for two hours. 4 ml of toluene were added and the reaction mixture was concentrated by evaporation *in vacuo* and dried. The residue was dissolved in 5 ml of water; 4 ml of a 2M HCl solution were added and the mixture was concentrated. The resulting tetrahydrochloride (**21a**) was dried *in vacuo* and suspended in 0.5 ml of DMF; 6 mmol of Ac_4(OMe)Neu5Ac-Gab-Ad-ONp (**8**) and 0.5 ml of NEt_3 were added and the mixture was stirred at room temperature for 24 hours. The reaction mixture was concentrated *in vacuo* and the product was purified by means of column chromatography (see Table 6). After drying *in vacuo*, the compound (**21**) was obtained in the form of a colourless amorphous product in a yield of 65%.

Compounds (**26**)-(**30**), (**32**)-(**36**) were prepared in an analogous manner (see Table 6).

[Ac_4(OMe)Neu5Ac-Gab-Ad-$NHCH_2$-]$_4$C (26). ^{1}H-NMR spectrum in D_6-DMSO (δ, ppm): matrix: 1.534 (m, 4H, 2 $COCH_2C\underline{H_2}$), 2.171 (m, 4H, 2 $COC\underline{H_2}CH_2$), 2.891 (br., 2H, CCH_2), 3.867 (d, 2H, $ArNHCOC\underline{H_2}$), 7.674 (br. t, 1H, $CCH_2N\underline{H}$), 8.107 (t, 1H, J 6 Hz, $N\underline{H}COCH_2CH_2$), 9.964 (s, 1H, ArNH); Neu5Acα2-$OCH_2C_6H_4$ fragment: 1.677, 1.918, 1.975, 2.024 and 2.094 (s, 15H, 5 $COCH_3$), 1.761 (dd, 1H, J_4 12.2 Hz, H-3ax), 2.570 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3eq), 3.697 (s, 3H, $COOCH_3$), 3.904 (ddd, 1H, J_4 10.3 Hz, H-5), 4.028 (dd, 1H, J_8 6.3 Hz, H-9b), 4.087 (dd, 1H, J_5 10.7 Hz, H-6), 4.233 (dd, 1H, J_{9b} 12.5 Hz, J_8 3 Hz, H-9a), 4.320 and 4.643 (d, 2H, J 11.8 Hz, $ArC\underline{H_2}$), 4.711 (ddd, 1H, H-4), 5.192 (dd, 1H, J_8 8.4 Hz, J_6 2.4 Hz, H-7), 5.341 (ddd, 1H, H-8), 7.214 and 7.555 (d, 2H, J 8.6 Hz, Ar), 7.708 (d, 1H, J_5 9.6 Hz, NH).

[Ac_4(OMe)Neu5Ac-Gab-Ad-Gly-$NHCH_2$-]$_4$C (27). ^{1}H-NMR spectrum in D_6-DMSO (δ, ppm): matrix: 1.509 (m, 4H, 2 $COCH_2C\underline{H_2}$), 2.147 and 2.231 (m, 4H, 2 $COC\underline{H_2}CH_2$), 2.674 (br., 2H, CCH_2), 3.647 (m, 2H, Gly), 3.859 (d, 2H, $ArNHCOC\underline{H_2}$), 7.852 (br. t, 1H, $CCH_2N\underline{H}$), 8.100 (t, 1H, J 6 Hz, $N\underline{H}COCH_2CH_2$), 8.453 (t, 1H, J 6 Hz, $N\underline{H}$Gly), 9.962 (s, 1H, ArNH). Neu5Acα2-$OCH_2C_6H_4$ fragment: 1.677, 1.918, 1.975, 2.024 and 2.094 (s, 15H, 5 $COCH_3$), 1.761 (dd, 1H, J_4 12.2 Hz, H-3ax), 2.570 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3eq), 3.697 (s, 3H,

$COOCH_3$), 3.904 (ddd, 1H, J_4 10.3 Hz, H-5), 4.028 (dd, 1H, J_8 6.3 Hz, H-9b), 4.087 (dd, 1H, J_5 10.7 Hz, H-6), 4.233 (dd, 1H, J_{9b} 12.5 Hz, J_8 3 Hz, H-9a), 4.320 and 4.643 (d, 2H, J 11.8 Hz, $ArCH_2$), 4.711 (ddd, 1H, H-4), 5.192 (dd, 1H, J_8 8.4 Hz, J_6 2.4 Hz, H-7), 5.341 (ddd, 1H, H-8), 7.214 and 7.555 (d, 2H, J 8.6 Hz, Ar), 7.708 (d, 1H, J_5 9.6 Hz, NH).

[Ac_4(OMe)Neu5Ac-Gab-Ad-Gly_2-$NHCH_2$-]$_4$C (28). ^{1}H-NMR spectrum in D_6-DMSO (δ, ppm): matrix: 1.495 (m, 4H, 2 $COCH_2CH_2$), 2.150 (m, 4H, 2 $COCH_2CH_2$), 2.694 (br., 2H, CCH_2), 3.716 (d, 2H, CH_2^{Gly2}), 3.818 (d, 2H, CH_2^{Gly1}), 3.865 (d, 2H, $ArNHCOCH_2$), 7.824 (br. t, 1H, CCH_2NH), 7.993 (t, 1H, J 6 Hz, NH^{Gly2}), 8.096 (t, 1H, J 6 Hz, $NHCOCH_2CH_2$), 8.544 (t, 1H, J 6 Hz, NH^{Gly1}), 9.975 (s, 1H, ArNH). Neu5Acα2-$OCH_2C_6H_4$ fragment: 1.677, 1.918, 1.975, 2.024 and 2.094 (s, 15H, 5 $COCH_3$), 1.761 (dd, 1H, J_4 12.2 Hz, H-3ax), 2.570 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3eq), 3.697 (s, 3H, $COOCH_3$), 3.904 (ddd, 1H, J_4 10.3 Hz, H-5), 4.028 (dd, 1H, J_8 6.3 Hz, H-9b), 4.087 (dd, 1H, J_5 10.7 Hz, H-6), 4.233 (dd, 1H, J_{9b} 12.5 Hz, J_8 3 Hz, H-9a), 4.320 and 4.643 (d, 2H, J 11.8 Hz, $ArCH_2$), 4.711 (ddd, 1H, H-4), 5.192 (dd, 1H, J_8 8.4 Hz, J_6 2.4 Hz, H-7), 5.341 (ddd, 1H, H-8), 7.214 and 7.555 (d, 2H, J 8.6 Hz, Ar), 7.708 (d, 1H, J_5 9.6 Hz, NH).

[Ac_4(OMe)Neu5Ac-Gab-Ad-Gly_3-$NHCH_2$]$_4$C (29). ^{1}H-NMR spectrum in D_6-DMSO (δ, ppm): matrix: 1.498 (m, 4H, 2 $COCH_2CH_2$), 2.143 and 2.158 (m, 4H, 2 $COCH_2CH_2$), 2.693 (br., 2H, CCH_2), 3.728 (m, 4H, 2 $CH_2^{Gly2,3}$), 3.841 (d, 2H, CH_2^{Gly1}), 3.862 (d, 2H, $ArNHCOCH_2$), 7.820 (br. t, 1H, CCH_2NH), 8.049 and 8.059 (t, 2H, J 5.7 Hz, $NH^{Gly2,3}$), 8.098 (t, 1H, J 5.8 Hz, $NHCOCH_2CH_2$), 8.547 (t, 1H, J 5.5 Hz, NH^{Gly1}), 9.972 (s, 1H, ArNH). Neu5Acα2-$OCH_2C_6H_4$ fragment: 1.677, 1.918, 1.975, 2.024 and 2.094 (s, 15H, 5 $COCH_3$), 1.761 (dd, 1H, J_4 12.2 Hz, H-3ax), 2.570 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3eq), 3.697 (s, 3H, $COOCH_3$), 3.904 (ddd, 1H, J_4 10.3 Hz, H-5), 4.028 (dd, 1H, J_8 6.3 Hz, H-9b), 4.087 (dd, 1H, J_5 10.7 Hz, H-6), 4.233 (dd, 1H, J_{9b} 12.5 Hz, J_8 3 Hz, H-9a), 4.320 and 4.643 (d, 2H, J 11.8 Hz, $ArCH_2$), 4.711 (ddd, 1H, H-4), 5.192 (dd, 1H, J_8 8.4 Hz, J_6 2.4 Hz, H-7), 5.341 (ddd, 1H, H-8), 7.214 and 7.555 (d, 2H, J 8.6 Hz, Ar), 7.708 (d, 1H, J_5 9.6 Hz, NH).

[Ac_4(OMe)Neu5Ac-Gab-Ad-Gly_4-$NHCH_2$]$_4$C (30).^{1}H-NMR spectrum in D_6-DMSO (δ, ppm), matrix: 1.500 (m, 4H, 2 $COCH_2CH_2$), 2.151 (m, 4H, 2 $COCH_2CH_2$), 2.688 (br., 2H, CCH_2), 3.720 (x2) and 3.753 (d, 6H, 3 CH_2^{Gly2-4}), 3.841 (d, 2H, CH_2^{Gly1}), 3.864 (d, 2H, $ArNHCOCH_2$), 7.818 (br. t, 1H, CCH_2NH), 8.045 and 8.084 (x2) (t, 3H, J 6 Hz, NH^{Gly2-4}), 8.102 (t, 1H, J 6 Hz, $NHCOCH_2CH_2$), 8.555 (t, 1H, J 5.5 Hz, NH^{Gly1}), 9.980 (s, 1H, ArNH). Neu5Acα2-$OCH_2C_6H_4$ fragment: 1.677, 1.918, 1.975, 2.024 and 2.094 (s, 15H, 5 $COCH_3$), 1.761 (dd, 1H, J_4 12.2 Hz, H-3ax), 2.570 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3eq), 3.697 (s, 3H, $COOCH_3$), 3.904 (ddd, 1H, J_4 10.3 Hz, H-5), 4.028 (dd, 1H, J_8 6.3 Hz, H-9b), 4.087 (dd, 1H, J_5 10.7 Hz, H-6), 4.233 (dd, 1H, J_{9b} 12.5 Hz, J_8 3 Hz, H-9a), 4.320 and 4.643 (d, 2H, J 11.8 Hz, $ArCH_2$), 4.711 (ddd, 1H, H-4), 5.192 (dd, 1H, J_8 8.4 Hz, J_6 2.4 Hz, H-7), 5.341 (ddd, 1H, H-8), 7.214 and 7.555 (d, 2H, J 8.6 Hz, Ar), 7.708 (d, 1H, J_5 9.6 Hz, NH).

[Ac_4(OMe)Neu5Ac-Gab-Ad-Gly_5-$NHCH_2$]$_4$C (31). ^{1}H-NMR spectrum in D_6-DMSO (δ, ppm): matrix: 1.502 (m, 4H, 2 $COCH_2C\underline{H}_2$), 2.147 and 2.159 (m, 4H, 2 $COC\underline{H}_2CH_2$), 2.688 (br., 2H, CCH_2), 3.738 (x2) and 3.765 (x2) (m, 8H, 4 CH_2^{Gly2-5}), 3.857 (d, 2H, CH_2^{Gly1}), 3.877 (d, 2H, $ArNHCOC\underline{H}_2$), 7.818 (br. t, 1H, $CCH_2N\underline{H}$), 8.074 (m, 5H, $N\underline{H}COCH_2CH_2$, $N\underline{H}^{Gly2-5}$), 8.551 (t, 1H, J 6 Hz, $N\underline{H}^{Gly1}$), 9.968 (s, 1H, ArNH). Neu5Acα2-$OCH_2C_6H_4$ fragment: 1.677, 1.918, 1.975, 2.024 and 2.094 (s, 15H, 5 $COCH_3$), 1.761 (dd, 1H, J_4 12.2 Hz, H-3ax), 2.570 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3eq), 3.697 (s, 3H, $COOCH_3$), 3.904 (ddd, 1H, J_4 10.3 Hz, H-5), 4.028 (dd, 1H, J_8 6.3 Hz, H-9b), 4.087 (dd, 1H, J_5 10.7 Hz, H-6), 4.233 (dd, 1H, J_{9b} 12.5 Hz, J_8 3 Hz, H-9a), 4.320 and 4.643 (d, 2H, J 11.8 Hz, $ArC\underline{H}_2$), 4.711 (ddd, 1H, H-4), 5.192 (dd, 1H, J_8 8.4 Hz, J_6 2.4 Hz, H-7), 5.341 (ddd, 1H, H-8), 7.214 and 7.555 (d, 2H, J 8.6 Hz, Ar), 7.708 (d, 1H, J_5 9.6 Hz, NH).

[Ac_4(OMe)Neu5Ac-Gab-Ad-AC_2-Gly_5-$NHCH_2$]$_4$C (32). ^{1}H-NMR spectrum in D_6-DMSO (δ, ppm), matrix: 1.224, 1.366 and 1.469 (m, 12H, 6 CH_2), 1.502 (m, 4H, 2 $COCH_2C\underline{H}_2$), 2.032 and 2.121 (m, 2 $COCH_2$),), 2.147 and 2.159 (m, 4H, 2 $COC\underline{H}_2CH_2$), 2.688 (br., 2H, CCH_2), 3.00 (m, 4H, 2 $C\underline{H}_2NHCO$), 3.738 (x2) and 3.765 (x2) (m, 8H, 4 CH_2^{Gly2-5}), 3.857 (d, 2H, CH_2^{Gly1}), 3.877 (d, 2H, $ArNHCOC\underline{H}_2$), 7.679 and 7.700 (br. t, 2H, 2 NHCO), 7.818 (br. t, 1H, $CCH_2N\underline{H}$), 8.074 (m, 5H, $N\underline{H}COCH_2CH_2$, $N\underline{H}^{Gly2-5}$), 8.551 (t, 1H, J 6 Hz, $N\underline{H}^{Gly1}$), 9.968 (s, 1H, ArNH). Neu5Acα2-$OCH_2C_6H_4$ fragment: 1.677, 1.918, 1.975, 2.024 and 2.094 (s, 15H, 5 $COCH_3$), 1.761 (dd, 1H, J_4 12.2 Hz, H-3ax), 2.570 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3eq), 3.697 (s, 3H, $COOCH_3$), 3.904 (ddd, 1H, J_4 10.3 Hz, H-5), 4.028 (dd, 1H, J_8 6.3 Hz, H-9b), 4.087 (dd, 1H, J_5 10.7 Hz, H-6), 4.233 (dd, 1H, J_{9b} 12.5 Hz, J_8 3 Hz, H-9a), 4.320 and 4.643 (d, 2H, J 11.8 Hz, $ArC\underline{H}_2$), 4.711 (ddd, 1H, H-4), 5.192 (dd, 1H, J_8 8.4 Hz, J_6 2.4 Hz, H-7), 5.341 (ddd, 1H, H-8), 7.214 and 7.555 (d, 2H, J 8.6 Hz, Ar), 7.708 (d, 1H, J_5 9.6 Hz, NH).

[Ac_4(OMe)Neu5Ac-Gab-Ad-AC_3-Gly_5-$NHCH_2$]$_4$C (33). ^{1}H-NMR spectrum in D_6-DMSO (δ, ppm) is very similar to the spectrum of compound (**32**) (the signals are in some cases broader and the integrals of the amidocaproic acid groups are correspondingly greater).

[Ac_4(OMe)Neu5Ac-Gab-AC-Ad-Gly_5-$NHCH_2$]$_4$C (34). ^{1}H-NMR spectrum in D_6-DMSO (δ, ppm): matrix: 1.250, 1.382, 1.465 and 1.506 (m, 10H, 5 CH_2), 2.033 and 2.140 (m, 6H, 3 $COCH_2$), 2.697 (br., 2H, CCH_2), 3.009 (m~q, 2H, J 6.4 Hz, $C\underline{H}_2NHCO$), 3.719 (x2) and 3.748 (x2) (m, 8H, 4 CH_2^{Gly2-5}), 3.843 (d, 2H, CH_2^{Gly1}), 3.862 (d, 2H, $ArNHCOC\underline{H}_2$), 4.327 and 4.648 (d, 2H, J 11.8 Hz, $ArC\underline{H}_2$), 7.216 and 7.555 (d, 2H, J 8 Hz, Ar), 7.698 (t, 1H, NHCO), 7.818 (br. t, 1H, $CCH_2N\underline{H}$), 8.039, 8.072, 8.084 (x2), 8.110 (m, 5H, $N\underline{H}COCH_2CH_2$, $N\underline{H}^{Gly2-5}$), 8.547 (t, 1H, $N\underline{H}^{Gly1}$), 9.970 (s, 1H, ArNH). Neu5Acα2-$OCH_2C_6H_4$ fragment: 1.677, 1.918, 1.975, 2.024 and 2.094 (s, 15H, 5 $COCH_3$), 1.761 (dd, 1H, J_4 12.2 Hz, H-3ax), 2.570 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3eq), 3.697 (s, 3H, $COOCH_3$), 3.904 (ddd, 1H, J_4 10.3 Hz, H-5), 4.028 (dd, 1H, J_8 6.3 Hz, H-9b), 4.087 (dd, 1H, J_5 10.7 Hz, H-6), 4.233 (dd, 1H, J_{9b} 12.5 Hz, J_8 3 Hz, H-9a), 4.320 and 4.643 (d, 2H, J 11.8 Hz, $ArC\underline{H}_2$), 4.711 (ddd, 1H, H-4), 5.192 (dd,

1H, J_8 8.4 Hz, J_6 2.4 Hz, H-7), 5.341 (ddd, 1H, H-8), 7.214 and 7.555 (d, 2H, J 8.6 Hz, Ar), 7.708 (d, 1H, J_5 9.6 Hz, NH).

[Ac_4(OMe)Neu5Ac-Gab-AC_2-Ad-Gly_5-$NHCH_2]_4C$ (35). ^{1}H-NMR spectrum in D_6-DMSO (δ, ppm): matrix: 1.239, 1.375, 1.465 and 1.509 (m, CH_2), 2.026 and 2.142 (m, $COCH_2$), 2.711 (br., 2H, CCH_2), 3.003 (m, 4H, 2 $C\underline{H}_2NHCO$), 3.718 (x2) and 3.746 (x2) (m, 8H, 4 CH_2^{Gly2-5}), 3.839 (d, 2H, CH_2^{Gly1}), 3.861 (d, 2H, $ArNHCOC\underline{H}_2$), 4.329 and 4.649 (d, 2H, J 11.8 Hz, $ArC\underline{H}_2$), 7.218 and 7.561 (d, 2H, J 8 Hz, Ar), 7.681 and 7.695 (m, 2H, 2 NHCO), 7.834 (br. t, 1H, $CCH_2N\underline{H}$), 8.077, 8.133 (x3), 8.177 (m, 5H, $N\underline{H}COCH_2CH_2$, $N\underline{H}^{Gly2-5}$), 8.587 (t, 1H, $N\underline{H}^{Gly1}$), 10.01 (s, 1H, ArNH). Neu5Acα2-$OCH_2C_6H_4$ fragment: 1.677, 1.918, 1.975, 2.024 and 2.094 (s, 15H, 5 $COCH_3$), 1.761 (dd, 1H, J_4 12.2 Hz, H-3ax), 2.570 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3eq), 3.697 (s, 3H, $COOCH_3$), 3.904 (ddd, 1H, J_4 10.3 Hz, H-5), 4.028 (dd, 1H, J_8 6.3 Hz, H-9b), 4.087 (dd, 1H, J_5 10.7 Hz, H-6), 4.233 (dd, 1H, J_{9b} 12.5 Hz, J_8 3 Hz, H-9a), 4.320 and 4.643 (d, 2H, J 11.8 Hz, $ArC\underline{H}_2$), 4.711 (ddd, 1H, H-4), 5.192 (dd, 1H, J_8 8.4 Hz, J_6 2.4 Hz, H-7), 5.341 (ddd, 1H, H-8), 7.214 and 7.555 (d, 2H, J 8.6 Hz, Ar), 7.708 (d, 1H, J_5 9.6 Hz, NH).

[Ac_4(OMe)Neu5Ac-Gab-AC_3-Ad-Gly_5-$NHCH_2]_4C$ (36). The ^{1}H-NMR spectrum in D_6-DMSO corresponds very substantially to the spectrum of compound (**35**), the signals are in some cases broader. Matrix (δ, ppm): 1.239, 1.375, 1.465 and 1.509 (m, CH_2), 2.026 and 2.142 (m, $COCH_2$), 2.629 (br., 2H, CCH_2), 3.00 (m, 6H, 3 $C\underline{H}_2NHCO$), 3.813 (br., 2H, CH_2^{Gly1}), 3.861 (d, 2H, $ArNHCOC\underline{H}_2$), 4.329 and 4.649 (d, 2H, J 11.8 Hz, $ArC\underline{H}_2$), 7.218 and 7.561 (d, 2H, J 8 Hz, Ar), 7.693 (m, 3H, 3 NHCO), 7.904 (br., 1H, $CCH_2N\underline{H}$), 8.083 (x2), 8.158 and 8.215 (x2) (m, 5H, $N\underline{H}COCH_2CH_2$, $N\underline{H}^{Gly2-5}$), 8.538 (t, 1H, $N\underline{H}^{Gly1}$). Neu5Acα2-$OCH_2C_6H_4$ fragment: 1.677, 1.918, 1.975, 2.024 and 2.094 (s, 15H, 5 $COCH_3$), 1.761 (dd, 1H, J_4 12.2 Hz, H-3ax), 2.570 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3eq), 3.697 (s, 3H, $COOCH_3$), 3.904 (ddd, 1H, J_4 10.3 Hz, H-5), 4.028 (dd, 1H, J_8 6.3 Hz, H-9b), 4.087 (dd, 1H, J_5 10.7 Hz, H-6), 4.233 (dd, 1H, J_{9b} 12.5 Hz, J_8 3 Hz, H-9a), 4.320 and 4.643 (d, 2H, J 11.8 Hz, $ArC\underline{H}_2$), 4.711 (ddd, 1H, H-4), 5.192 (dd, 1H, J_8 8.4 Hz, J_6 2.4 Hz, H-7), 5.341 (ddd, 1H, H-8), 7.214 and 7.555 (d, 2H, J 8.6 Hz, Ar), 7.708 (d, 1H, J_5 9.6 Hz, NH).

Table 6. Preparation of protected tetrasialosides (**26**)-(**36**) (Example 5)

End products	Matrix	Glycoside	Conditions	TLC		Isolation of the products	Yield %
				Rf	Eluant		
Methyl ester peracetate							
[Ac_4(OMe)Neu5Ac-Gab-Ad-$NHCH_2$-l4C (**26**)	(1)	6 mM (8)	DMF, 24 hours stirring at room temperature	0.21	C	Reaction mixture was concentrated by evaporation *in vacuo*; LC: $CHCl_3$/MeOH 10:1	57
[Ac_4(OMe)Neu5Ac-Gab-Ad-Gly-$NHCH_2$-l4C (**27**)	(17)			0.51	E	Reaction mixture was concentrated by evaporation *in vacuo*; LC: *i*-PrOH/EtOAc/H_2O 2:5:1	66
[Ac_4(OMe)Neu5Ac-Gab-Ad-Gly_2-$NHCH_2$-l4C (**28**)	(18)			0.25	E	Reaction mixture was concentrated by evaporation *in vacuo*; LC: acetone/MeOH/H_2O 20:1:1→5:1:1	63
[Ac_4(OMe)Neu5Ac-Gab-Ad-Gly_3-$NHCH_2$-l4C (**29**)	(19)			0.23	E	Reaction mixture was concentrated by evaporation *in vacuo*; LC: acetone/MeOH/H_2O 30:1:1→10:1:1	33
[Ac_4(OMe)Neu5Ac-Gab-Ad-Gly_4-$NHCH_2$-l4C (**30**)	(20)			0.40	G	Reaction mixture was concentrated by evaporation *in vacuo*; LC: acetone/MeOH/H_2O 15:1:1→5:1:1	68
[Ac_4(OMe)Neu5Ac-Gab-Ad-Gly_5-$NHCH_2$-l4C (**31**)	(21)			0.18	G	Reaction mixture was concentrated by evaporation *in vacuo*; LC: acetone/MeOH/H_2O 20:1:1→5:1:1	65
[Ac_4(OMe)Neu5Ac-Gab-Ad-AC_2-Gly_5-$NHCH_2$-l4C (**32**)	(24)	12 mM (8)	DMSO, 70°C 72 hours	0.15	G	Sephadex LH-20, MeCN/H_2O 1:1	60
[Ac_4(OMe)Neu5Ac-Gab-Ad-AC_3-Gly_5-$NHCH_2$-l4C (**33**)	(25)			0.45	H		57
[Ac_4(OMe)Neu5Ac-Gab-AC-Ad-Gly_5-$NHCH_2$-l4C (**34**)	(21)	12 mM (9)		0.16	G	Reaction mixture was lyophilised; LC: eluant G, then *i*-PrOH/MeOH/EtOAc/H_2O 4:3:3:3	76
[Ac_4(OMe)Neu5Ac-Gab-AC_2-Ad-Gly_5-$NHCH_2$-l4C (**35**)	(21)	12 mM (10)		0.11	G	Reaction mixture was lyophilised, LC: eluant G, then H	46
[Ac_4(OMe)Neu5Ac-Gab-AC_3-Ad-Gly_5-$NHCH_2$-l4C (**36**)	(21)	12 mM (11)		0.84	H		11

Example 6.

Preparation of free tetrasialosides

Preparation of *[Neu5Acα-$OCH_2(p\text{-}C_6H_4)NHCOCH_2NH\text{-}CO(CH_2)_4CO\text{-}(NH(CH_2)_5CO)_3\text{-}(NHCH_2CO)_5\text{-}NHCH_2\text{-}]_4C$ (ammonium salt)*

Neu5Ac-Gab-Ad-AC_3-Gly_5-$NHCH_2]_4C$ (44).

80 µl of 2N NaOH solution were added to a solution of 10 µmol of the protected tetrasialoside (**33**) in 3 ml of absolute MeOH, and after 3 hours 1.5 ml of water and 80 µl of 2N NaOH solution were again added. The mixture was stirred at room temperature overnight; 80 µl of AcOH were added and the mixture was evaporated to dryness. The product was obtained by means of gel chromatography over Sephadex G-10 using a 0.05M aqueous NH_4OH solution.
(see Table 7).

Compounds (**37**)-(**43**), (**45**)-(**47**) were obtained in an analogous manner (see Table 7).

Table 7 (Example 6)

Tetrasialosides		Starting compound	TLC, eluant H, R_f	Aggregate, %*	Yield %
[Neu5Ac-Gab-Ad-$NHCH_2$-]$_4$C	**(37)**	**(26)**	0.80	No self-association	76
[Neu5Ac-Gab-Ad-Gly-$NHCH_2$-]$_4$C	**(38)**	**(27)**	0.82		81
[Neu5Ac-Gab-Ad-Gly_2-$NHCH_2$-]$_4$C	**(39)**	**(28)**	0.81		91
[Neu5Ac-Gab-Ad-Gly_3-$NHCH_2$-]$_4$C	**(40)**	**(29)**	0.77		90
[Neu5Ac-Gab-Ad-Gly_4-$NHCH_2$-]$_4$C	**(41)**	**(30)**	0.75		83
[Neu5Ac-Gab-Ad-Gly_5-$NHCH_2$-]$_4$C	**(42)**	**(31)**	0.71		83
[Neu5Ac-Gab-Ad-AC_2-Gly_5-$NHCH_2$-]$_4$C	**(43)**	**(32)**	Monomer and aggregate are eluted separately;	6	87
[Neu5Ac-Gab-Ad-AC_3-Gly_5-$NHCH_2$-]$_4$C	**(44)**	**(33)**	monomer: $R_f \approx 0.6$	54	90
[Neu5Ac-Gab-AC-Ad-Gly_5-$NHCH_2$-]$_4$C	**(45)**	**(34)**	aggregate: $R_f \approx 0$	12	93
[Neu5Ac-Gab-AC_2-Ad-Gly_5-$NHCH_2$-]$_4$C	**(46)**	**(35)**		92	86
[Neu5Ac-Gab-AC_3-Ad-Gly_5-$NHCH_2$-]$_4$C	**(47)**	**(36)**		96	89

*Determined by means of gel permeation chromatography

[Neu5Ac-Gab-Ad-NHCH$_2$-]$_4$C (37). ^{1}H-NMR spectrum in D_2O (δ, ppm): matrix: 1.633 (m, 4H, $COCH_2C\underline{H_2}$), 2.293 and 2.358 (m, 4H, 2 $COC\underline{H_2}CH_2$), 2.943 (s, 2H, CCH_2), 4.003 (s, 2H, $ArNHCOC\underline{H_2}$), 4.493 and 4.718 (d, 2H, J 11 Hz, $ArCH_2$), 7.388 (m, 4H, Ar). Neu5Acα fragment : 1.680 (dd, 1H, J_4 12 Hz, H-3ax), 2.036 (s, 3H, NAc), 2.778 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3eq), 3.598 (dd, 1H, J_8 9 Hz, H-7), 3.636 (dd, 1H, J_8 6 Hz, H-9b), 3.695 (ddd, 1H, J_5 9.8 Hz, H-4), 3.728 (dd, 1H, J_7 1.5 Hz, J_5 10.2 Hz, H-6), 3.782 (ddd, 1H, H-8), 3.822 (dd, 1H, H-5), 3.846, (dd, 1H, J_{9b} 12 Hz, J_8 2.3 Hz, H-9a).

[Neu5Ac-Gab-Ad-Gly-NHCH$_2$-]$_4$C (38). ^{1}H-NMR spectrum in D_2O (δ, ppm): matrix: 1.622 (m, 4H, $COCH_2C\underline{H_2}$), 2.340 and 2.382 (m, 4H, 2 $COC\underline{H_2}CH_2$), 2.810 (s, 2H, CCH_2), 3.847 (s, 2H, CH_2^{Gly}), 4.016 (s, 2H, $ArNHCOC\underline{H_2}$), 4.492 and 4.707 (d, 2H, J 11 Hz, $ArCH_2$), 7.402 (m, 4H, Ar). Neu5Acα fragment : 1.680 (dd, 1H, J_4 12 Hz, H-3ax), 2.036 (s, 3H, NAc), 2.778 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3eq), 3.598 (dd, 1H, J_8 9 Hz, H-7), 3.636 (dd, 1H, J_8 6 Hz, H-9b), 3.695 (ddd, 1H, J_5 9.8 Hz, H-4), 3.728 (dd, 1H, J_7 1.5 Hz, J_5 10.2 Hz, H-6), 3.782 (ddd, 1H, H-8), 3.822 (dd, 1H, H-5), 3.846, (dd, 1H, J_{9b} 12 Hz, J_8 2.3 Hz, H-9a).

[Neu5Ac-Gab-Ad-Gly$_2$-NHCH$_2$-]$_4$C (39). ^{1}H-NMR spectrum in D_2O (δ, ppm): matrix: 1.626 (m, 4H, $COCH_2C\underline{H_2}$), 2.341 (m, 4H, 2 $COC\underline{H_2}CH_2$), 2.831 (s, 2H, CCH_2), 3.894 and 3.991 (s, 4H, 2 $CH_2^{Gly1,2}$), 4.022 (s, 2H, $ArNHCOC\underline{H_2}$), 4.492 and 4.719 (d, 2H, J 11 Hz, $ArCH_2$), 7.402 (m, 4H, Ar). Neu5Acα fragment : 1.680 (dd, 1H, J_4 12 Hz, H-3ax), 2.036 (s, 3H, NAc), 2.778 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3eq), 3.598 (dd, 1H, J_8 9 Hz, H-7), 3.636 (dd, 1H, J_8 6 Hz, H-9b), 3.695 (ddd, 1H, J_5 9.8 Hz, H-4), 3.728 (dd, 1H, J_7 1.5 Hz, J_5 10.2 Hz, H-6), 3.782 (ddd, 1H, H-8), 3.822 (dd, 1H, H-5), 3.846, (dd, 1H, J_{9b} 12 Hz, J_8 2.3 Hz, H-9a).

[Neu5Ac-Gab-Ad-Gly$_3$-NHCH$_2$-]$_4$C (40). ^{1}H-NMR spectrum in D_2O (δ, ppm): matrix: 1.631 (m, 4H, $COCH_2C\underline{H_2}$), 2.344 (m, 4H, 2 $COC\underline{H_2}CH_2$), 2.857 (s, 2H, CCH_2), 3.912, 3.931 and 4.024 (s, 6H, 3 CH_2^{Gly1-3}), 4.029 (s, 2H, $ArNHCOC\underline{H_2}$), 4.500 and 4.725 (d, 2H, J 11 Hz, $ArCH_2$), 7.408 (m, 4H, Ar). Neu5Acα fragment : 1.680 (dd, 1H, J_4 12 Hz, H-3ax), 2.036 (s, 3H, NAc), 2.778 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3eq), 3.598 (dd, 1H, J_8 9 Hz, H-7), 3.636 (dd, 1H, J_8 6 Hz, H-9b), 3.695 (ddd, 1H, J_5 9.8 Hz, H-4), 3.728 (dd, 1H, J_7 1.5 Hz, J_5 10.2 Hz, H-6), 3.782 (ddd, 1H, H-8), 3.822 (dd, 1H, H-5), 3.846, (dd, 1H, J_{9b} 12 Hz, J_8 2.3 Hz, H-9a).

[Neu5Ac-Gab-Ad-Gly$_4$-NHCH$_2$-]$_4$C (41). ^{1}H-NMR spectrum in D_2O (δ, ppm): matrix: 1.636 (m, 4H, $COCH_2C\underline{H_2}$), 2.350 (m, 4H, 2 $COC\underline{H_2}CH_2$), 2.864 (s, 2H, CCH_2), 3.912, 3.934, 3.968 and 4.025 (s, 8H, 4 CH_2^{Gly1-4}), 4.032 (s, 2H, $ArNHCOC\underline{H_2}$), 4.497 and 4.725 (d, 2H, J 11 Hz, $ArCH_2$), 7.408 (m, 4H, Ar). Neu5Acα fragment : 1.680 (dd, 1H, J_4 12 Hz, H-3ax), 2.036 (s, 3H, NAc), 2.778 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3eq), 3.598 (dd, 1H, J_8 9 Hz, H-7), 3.636 (dd, 1H, J_8 6 Hz, H-9b), 3.695 (ddd, 1H, J_5 9.8 Hz, H-4), 3.728 (dd, 1H, J_7 1.5 Hz, J_5 10.2 Hz, H-6), 3.782 (ddd, 1H, H-8), 3.822 (dd, 1H, H-5), 3.846, (dd, 1H, J_{9b} 12 Hz, J_8 2.3 Hz, H-9a).

[Neu5Ac-Gab-Ad-Gly$_5$-NHCH$_2$-]$_4$C (42). ^{1}H-NMR spectrum in D_2O (δ, ppm): matrix: 1.638 (m, 4H, $COCH_2C\underline{H_2}$), 2.355 (m, 4H, 2 $CO\underline{CH_2}CH_2$), 2.878 (s, 2H, CCH_2), 3.921, 3.933, 3.974 (x2) and 4.032 (s, 10H, 5 CH_2^{Gly1-5}), 4.036 (s, 2H, $ArNHCOC\underline{H_2}$), 4.502 and 4.724 (d, 2H, J 11 Hz, $ArCH_2$), 7.410 (m, 4H, Ar). Neu5Acα fragment : 1.680 (dd, 1H, J_4 12 Hz, H-3ax), 2.036 (s, 3H, NAc), 2.778 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3eq), 3.598 (dd, 1H, J_8 9 Hz, H-7), 3.636 (dd, 1H, J_8 6 Hz, H-9b), 3.695 (ddd, 1H, J_5 9.8 Hz, H-4), 3.728 (dd, 1H, J_7 1.5 Hz, J_5 10.2 Hz, H-6), 3.782 (ddd, 1H, H-8), 3.822 (dd, 1H, H-5), 3.846, (dd, 1H, J_{9b} 12 Hz, J_8 2.3 Hz, H-9a).

[Neu5Ac-Gab-Ad-AC$_2$-Gly$_5$-NHCH$_2$-]$_4$C (43). ^{1}H-NMR spectrum in D_2O (δ, ppm): matrix: 1.286, 1.476 and 1.567 (m, 12H, 6 CH_2), 1.623 (m, 4H, $COCH_2C\underline{H_2}C\underline{H_2}CH_2CO$), 2.179 (t, 2H, J 7.4 Hz, CH_2CO), 2.245 and 2.367 (m, 4H, $CO\underline{CH_2}CH_2CH_2C\underline{H_2}CO$), 2.299 (t, 2H, J 7.4 Hz, CH_2CO), 2.882 (s, 2H, CCH_2), 3.133 (m, 4H, 2 CH_2N), 3.928, 3.940, 3.987 (x2) and 4.043 (x2) (s, 12H, 6 $COCH_2N$), 4.502 and 4.730 (d, 2H, J 11 Hz, $ArCH_2$), 7.418 (m, 4H, Ar). Neu5Acα fragment : 1.680 (dd, 1H, J_4 12 Hz, H-3ax), 2.036 (s, 3H, NAc), 2.778 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3eq), 3.598 (dd, 1H, J_8 9 Hz, H-7), 3.636 (dd, 1H, J_8 6 Hz, H-9b), 3.695 (ddd, 1H, J_5 9.8 Hz, H-4), 3.728 (dd, 1H, J_7 1.5 Hz, J_5 10.2 Hz, H-6), 3.782 (ddd, 1H, H-8), 3.822 (dd, 1H, H-5), 3.846, (dd, 1H, J_{9b} 12 Hz, J_8 2.3 Hz, H-9a).

Aggregate **[Neu5Ac-Gab-Ad-AC$_3$-Gly$_5$-NHCH$_2$-]$_4$C (44).** ^{1}H-NMR spectrum in D_2O is very similar to the spectrum of compound (**43**),
matrix (δ, ppm): 1.283, 1.476, 1.570 (m, 18H, 9 CH_2), 2.178 and 2.189 (t, 2x2H, J 7.4 Hz, 2 CH_2CO), 2.301 (t, 2H, J 7.4 Hz, CH_2CO), 3.135 (m, 6H, 3 CH_2N), 3.928, 3.940, 3.987 (x2) and 4.043 (x2) (s, 12H, 6 $COCH_2N$), 4.502 and 4.730 (d, 2H, J 11 Hz, $ArCH_2$), 7.418 (m, 4H, Ar).
Neu5Acα fragment : 1.680 (dd, 1H, J_4 12 Hz, H-3ax), 2.036 (s, 3H, NAc), 2.778 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3eq), 3.598 (dd, 1H, J_8 9 Hz, H-7), 3.636 (dd, 1H, J_8 6 Hz, H-9b), 3.695 (ddd, 1H, J_5 9.8 Hz, H-4), 3.728 (dd, 1H, J_7 1.5 Hz, J_5 10.2 Hz, H-6), 3.782 (ddd, 1H, H-8), 3.822 (dd, 1H, H-5), 3.846, (dd, 1H, J_{9b} 12 Hz, J_8 2.3 Hz, H-9a).

[Neu5Ac-Gab-AC-Ad-Gly$_5$-NHCH$_2$-]$_4$C (45). ^{1}H-NMR spectrum in D_2O (δ, ppm): matrix: 1.334 (m, 2H, CH_2), 1.504 (m, 2H, $C\underline{H_2}CH_2NH$), 1.569 (m, 4H, $COCH_2C\underline{H_2}C\underline{H_2}CH_2CO$), 1.625 (m, 2H, $C\underline{H_2}CH_2CO$), 2.207 and 2.313 (m, 4H, $CO\underline{CH_2}CH_2CH_2C\underline{H_2}CO$), 2.344 (t, 2H, J 7 Hz, CH_2CO), 2.885 (s, 2H, CCH_2), 3.156 (t, 2H, J 7.4 Hz, CH_2N), 3.928, 3.942, 3.979, 3.984, 4.037 and 4.042 (s, 12H, 6 $COCH_2N$), 4.506 and 4.729 (d, 2H, J 11 Hz, $ArCH_2$), 7.420 (m, 4H, Ar). Neu5Acα fragment : 1.680 (dd, 1H, J_4 12 Hz, H-3ax), 2.036 (s, 3H, NAc), 2.778 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3eq), 3.598 (dd, 1H, J_8 9 Hz, H-7), 3.636 (dd, 1H, J_8 6 Hz, H-9b), 3.695 (ddd, 1H, J_5 9.8 Hz, H-4), 3.728 (dd, 1H, J_7 1.5 Hz, J_5 10.2 Hz, H-6), 3.782 (ddd, 1H, H-8), 3.822 (dd, 1H, H-5), 3.846, (dd, 1H, J_{9b} 12 Hz, J_8 2.3 Hz, H-9a).

Aggregate **[Neu5Ac-Gab-AC$_2$-Ad-Gly$_5$-NHCH$_2$-]$_4$C (46).** ^{1}H-NMR spectrum in D_2O (δ, ppm): matrix: 1.268, 1.504 and 1.630 (m, 12H, 6 CH_2), 1.572 (m, 4H, $COCH_2C\underline{H_2}C\underline{H_2}CH_2CO$), 2.185 (t, 2H, J 7 Hz, CH_2CO), 2.212 and 2.315 (m, 4H, $COC\underline{H_2}CH_2CH_2C\underline{H_2}CO$), 2.349 (t, 2H, J 7.4 Hz, CH_2CO), 2.898 (s, 2H, CCH_2), 3.130 and 3.158 (t, 2x2H, J 7.4 Hz, 2 CH_2N), 3.934, 3.945, 3.987 (x2), 4.039 and 4.045 (s, 12H, 6 $COCH_2N$), 4.509 and 4.725 (d, 2H, J 11 Hz, $ArCH_2$), 7.422 (m, 4H, Ar). Neu5Acα fragment : 1.680 (dd, 1H, J_4 12 Hz, H-3ax), 2.036 (s, 3H, NAc), 2.778 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3eq), 3.598 (dd, 1H, J_8 9 Hz, H-7), 3.636 (dd, 1H, J_8 6 Hz, H-9b), 3.695 (ddd, 1H, J_5 9.8 Hz, H-4), 3.728 (dd, 1H, J_7 1.5 Hz, J_5 10.2 Hz, H-6), 3.782 (ddd, 1H, H-8), 3.822 (dd, 1H, H-5), 3.846, (dd, 1H, J_{9b} 12 Hz, J_8 2.3 Hz, H-9a).

Aggregate **[Neu5Ac-Gab-AC$_3$-Ad-Gly$_5$-NHCH$_2$-]$_4$C (47).** The ^{1}H-NMR spectrum in D_2O is very similar to the spectrum of compound (**46**), the signals are in some cases broader. Matrix (δ, ppm): 1.276, 1.461 and 1.630 (m, 18H, 9 CH_2), 2.186 (t, 2x2H, J 7 Hz, 2 CH_2CO), 2.349 (t, 2H, J 7.4 Hz, CH_2CO), 3.132 (m, 6H, 3 CH_2N), 3.934, 3.945, 3.987 (x2), 4.039 and 4.045 (s, 12H, 6 $COCH_2N$), 4.509 and 4.725 (d, 2H, J 11 Hz, $ArCH_2$), 7.422 (m, 4H, Ar). Neu5Acα fragment : 1.680 (dd, 1H, J_4 12 Hz, H-3ax), 2.036 (s, 3H, NAc), 2.778 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3eq), 3.598 (dd, 1H, J_8 9 Hz, H-7), 3.636 (dd, 1H, J_8 6 Hz, H-9b), 3.695 (ddd, 1H, J_5 9.8 Hz, H-4), 3.728 (dd, 1H, J_7 1.5 Hz, J_5 10.2 Hz, H-6), 3.782 (ddd, 1H, H-8), 3.822 (dd, 1H, H-5), 3.846, (dd, 1H, J_{9b} 12 Hz, J_8 2.3 Hz, H-9a).

Example 7.

Preparation of the aggregate *{[Neu5Acα-OCH$_2$(p-C$_6$H$_4$)NHCOCH$_2$NH-CO(CH$_2$)$_4$CO-(NHCH$_2$CO)$_7$-NHCH$_2$-]$_4$C}$_x$ (ammonium salt)*

{[Neu5Ac-Gab-Ad-Gly$_7$-NHCH$_2$-]$_4$C}$_x$ (48).

18.8 mg (26 µmol) of the lyophilised compound (**15**) are added to 6.1 mg (3.25 µmol) of tetrahydrochloride (**22a**), prepared as described in Example 4, in 0.5 ml of water. The pH of the reaction mixture was adjusted to pH = 8 with 1M $NaHCO_3$ solution. The reaction solution was stirred at room temperature for 3 days, the pH being maintained at 8 by the addition of 1M $NaHCO_3$ solution. The reaction mixture was separated over a Sephadex LH-20 column with a 0.05M aqueous NH_4OH solution. After concentration and drying *in vacuo*, 9.6 mg of product (**48**) were obtained, corresponding to a yield of 71%.

^{1}H-NMR spectrum (D_2O, δ, ppm): matrix: 1.638 (m, 4H, $COCH_2C\underline{H_2}$), 2.358 (m, 4H, 2 $COC\underline{H_2}CH_2$), 2.878 (s, 2H, CCH_2), 3.918, 3.938, 3.978 (x4) and 4.034 (s, 14H, 7 CH_2^{Gly1-7}), 4.037 (s, 2H, $ArNHCOC\underline{H_2}$), 4.498 and 4.718 (d, 2H, J 11 Hz, $ArCH_2$), 7.408 (m, 4H, Ar). Neu5Acα fragment : 1.680 (dd, 1H, J_4 12 Hz, H-3ax), 2.036 (s, 3H, NAc), 2.778 (dd, 1H, J_{3ax}

12.5 Hz, J_4 4.6 Hz, H-3eq), 3.598 (dd, 1H, J_8 9 Hz, H-7), 3.636 (dd, 1H, J_8 6 Hz, H-9b), 3.695 (ddd, 1H, J_5 9.8 Hz, H-4), 3.728 (dd, 1H, J_7 1.5 Hz, J_5 10.2 Hz, H-6), 3.782 (ddd, 1H, H-8), 3.822 (dd, 1H, H-5), 3.846, (dd, 1H, J_{9b} 12 Hz, J_8 2.3 Hz, H-9a).

Example 8.

Preparation of aggregates

Preparation of *{Galα1-3Galβ1-O(CH$_2$)$_3$NH-CO(CH$_2$)$_4$CO-(NH(CH$_2$)$_5$CO)$_3$-(NHCH$_2$CO)$_5$-NHCH$_2$-]$_4$C}$_x$*

{[B_{di}-Ap-Ad-AC_3-Gly_5-$NHCH_2$-]$_4$C}$_x$ (**49**).

15.6 mg of (**16**) and 5 µl of Et_3N were added to a suspension von 5.6 mg (2 µmol) of tetrahydrochloride (**25a**), prepared as described in Example 4, in 0.5 ml of DMSO. The reaction solution was stirred at 40°C for 3 days. After the addition of 0.2 ml of conc. NH_4OH solution, the reaction mixture was stirred for 30 minutes and separated over a Sephadex LH-20 column with MeCN/H_2O 1:1. After concentration and drying *in vacuo*, 6.4 mg of product (**49**) were obtained, corresponding to a yield of 69%.

^{1}H-NMR spectrum (D_2O/CD_3OD 2:1, δ, ppm): 1.374, 1.562 and 1.645 (m, CH_2), 1.883 (m, 2H, $OCH_2C\underline{H}_2CH_2N$), 2.265 (t, 4H, J 7.5 Hz, 2 CH_2CO), 2.292 (m, 4H, 2 CH_2CO), 2.377 (t, 2H, J 7.5 Hz, CH_2CO), 2.955 (br. s, CCH_2), 3.213 (t, 6H, 3 CH_2N), 3.348 (m, 2H, $OCH_2CH_2C\underline{H}_2N$), 3.697 (dd, 1H, H-2 Galβ), 3.756 (m, $OC\underline{H}CH_2CH_2N$), 3.910 (dd, 1H, J_3 10 Hz, H-2 Galα), 4.00, 4.046 and 4.097 (s, 10H, 5 $COCH_2N$), 4.205 (d, 1H, J_3 3 Hz, H-4 Galβ), 4.255 (m, 1H, H-5 Galα), 4.462 (d, 1H, J_2 8 Hz, H-1 Galβ), 5.184 (d, 1H, J_2 4 Hz, H-1 Galα).

Preparation of *{[Neu5Acα2-3Galβ1-4Glcβ1-NHCOCH$_2$NH-CO(CH$_2$)$_4$CO-(NHCH$_2$CO)$_5$-NHCH$_2$-]$_4$C}$_x$*

{[3`SL-NHCOCH$_2$NH-Ad-Gly$_5$-NHCH$_2$-]$_4$C}$_x$ (50)

was prepared starting from (**21a**) and (**14**) analogously to compound (**49**).

TLC: R_f 0.52 (methanol/acetonitrile/water 6:6:3). Yield 65%.

^{1}H-NMR spectrum (D_2O, δ, ppm): 1.622 (m, 4H, $C\underline{H}_2C\underline{H}_2CH_2CO$), 1.797 (dd, 1H, J_4 12 Hz, H-3_{ax} Neu5Ac), 2.017 (s, 3H, $COCH_3$), 2.342 (m, 4H, 2 CH_2CO), 2.744 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3_{eq} Neu5Ac), 2.895 (br. s, CCH_2), 3.452 (dd, 1H, H-2 Glcβ), 3.568 (dd, 1H, J_3 10 Hz, H-2 Galβ), 3.954, 3.992 and 4.041 (s, 12H, 6 $COCH_2N$), 4.105 (dd, 1H, J_2 10 Hz, J_4 3 Hz, H-3 Galβ), 4.523 (d, 1H, J_2 8 Hz, H-1 Galβ), 5.005 (d, 1H, J_2 9 Hz, H-1 Glcβ).

Preparation of *{[Neu5Acα2-3Galβ1-4Glcβ1-NHCOCH$_2$NH-CO(CH$_2$)$_4$CO-(NHCH$_2$CO)$_7$-NHCH$_2$-]$_4$C }$_x$*

{[3`SL-NHCOCH$_2$NH-Ad-Gly$_7$-NHCH$_2$-]$_4$C}$_x$ (51)

was prepared analogously to compound (**48**) starting from (**22a**) and (**14**). Yield 78%. ^{1}H-NMR spectrum (D_2O, δ, ppm): 1.622 (m, 4H, C$\underline{H_2}$C$\underline{H_2}$CH$_2$CO), 1.797 (dd, 1H, J_4 12 Hz, H-3$_{ax}$ Neu5Ac), 2.017 (s, 3H, COCH$_3$), 2.342 (m, 4H, 2 CH$_2$CO), 2.744 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3$_{eq}$ Neu5Ac), 2.895 (br. s, CCH$_2$), 3.452 (dd, 1H, H-2 Glcβ), 3.568 (dd, 1H, J_3 10 Hz, H-2 Galβ), 3.954, 3.992 and 4.041 (s, 16H, 8 COCH$_2$N), 4.105 (dd, 1H, J_2 10 Hz, J_4 3 Hz, H-3 Galβ), 4.523 (d, 1H, J_2 8 Hz, H-1 Galβ), 5.005 (d, 1H, J_2 9 Hz, H-1 Glcβ).

Example 9

Induction of self-association of [HCl·H-Gly$_7$-NHCH$_2$-]$_4$C (**22a**).

The investigation into the light scattering of a 50mM solution of compound (**22a**) in water was carried out using a Spectra-Physics 164 argon laser (plasma lines λ=528.7 and 611.5 nm), the scattering was measured at an angle of 90° to the incident light beam. The particle size thereby determined was <2.5 nm. 50 µl of a 0.8M NaHCO$_3$ solution were added to the resulting solution. The light scattering was meausured, as described above, the average particle size thereby determined was 200-400 nm.

50 µl of a 0.8M HCl was added to the resulting solution, and the sample was investigated by means of light scattering, as described above. The particle size thereby determined was < 2.5 nm.

Example 10

Inhibition of the viral cell adhesion of influenza viruses

The specific binding constants of the inhibitor virus complexes were determined by means of a fetuin binding assay, as described in the literature (US Patent 5,571,836, 1996; PCT WO 98/14215).

Table 8, Example 10
Influenza virus A/NIB/44/90M H3N2

Inhibitor	Compound No.	K_d, µM
Neu5Acα-OBn		100
[Neu5Ac-Gab-Ad-Gly_n-$NHCH_2$-]$_4$C (n=0-5)	(**38**) - (**42**)	~50
[Neu5Ac-Gab-Ad-Gly_7-$NHCH_2$-]$_4$C	(**48**)	0.1
[Neu5Ac-Ap-Ad-Gly_n-$NHCH_2$-]$_4$C (n=0-3)		200
[Neu5Ac-Gab-AC-Ad-Gly_5-$NHCH_2$-]$_4$C	(**45**)	7
[Neu5Ac-Gab-AC_2-Ad-Gly_5-$NHCH_2$-]$_4$C	(**46**)	0.3
[Neu5Ac-Gab-AC_3-Ad-Gly_5-$NHCH_2$-]$_4$C	(**47**)	0.1
[Neu5Ac-Gab-Ad-AC_2-Gly_5-$NHCH_2$-]$_4$C	(**43**)	0.1
[Neu5Ac-Gab-Ad-AC_3-Gly_5-$NHCH_2$-]$_4$C	(**44**)	0.04
Influenza virus A/Duck/Alberta/60/67 H12N5		
3`SL		20
[3`SL-$NHCOCH_2NH$-Ad-Gly_5-$NHCH_2$-]$_4$C	(**50**)	1
[3`SL-$NHCOCH_2NH$-Ad-Gly_7-$NHCH_2$-]$_4$C	(**51**)	0.1

Example 11

Inhibition of the complement-dependent cytotoxicity of human blood sera with respect to PK 15 cells as a result of the aggregate *{[B_{di}-Ap-Ad-AC_3-Gly_5-$NHCH_2$-]$_4$C}$_x$* (**49**)

Serial dilutions of the B disaccharide Galα1-3Gal and of the aggregate *{[B_{di}-Ap-Ad-AC_3-Gly_5-$NHCH_2$-]$_4$C}$_x$* (**49**) with human blood serum were incubated at 4°C overnight, and the inhibition of the cytotoxicity was demonstrated, as described in the literature (R.Rieben, E.von Allmen, E.Y.Korchagina, U.E.Nydegger, F.A.Neethling, M.Kujundzic, E.Koren, N.V.Bovin, D.K.C.Cooper, *Xenotransplantation*, 2, 98, 1995). After the addition of the complement constituents in the form of 10% rabbit serum (Sigma), the samples were incubated for 10 minutes with PK15 cells grown on Terasaki plates. The cells were then washed and stained using a cytotoxicity kit ("live/dead" viability/cytotoxicity kit, Molecular Probes, Eugene, OR, USA). By measuring the fluorescence intensities, the live/dead proportions were determined. The inhibition of cytotoxicity was calculated by comparison with a serum sample to which no inhibitor had been added. In the case of the following concentrations (calculated as molar concentration of the B disaccharide units), 50 % inhibition of cytotoxicity was achieved:

Galα1-3Gal (B disaccharide)	200 µM
{[B_{di}-Ap-Ad-AC_3-Gly_5-$NHCH_2$-]$_4$C}$_x$ aggregate (**49**)	0.5 µM

Example 12

The divalent matrices of the formula **[HCl·H-Gly$_n$-NHCH$_2$CH$_2$-]$_2$** (n = 2, 4) were prepared starting from 1,4-diaminobutane analogously to the sythesis in Example 4.

Preparation of *bis-1,4-(hexaglycilamido)-butane* **[HCl·H-Gly$_6$-NHCH$_2$CH$_2$-]$_2$** (52).

48 mg of BocGlyGlyNOS (146 μmol) and 0.1 ml of Et_3N were added to a solution of 30 mg of the compound **[HCl·H-Gly$_4$-NHCH$_2$CH$_2$-]$_2$** (48.6 μmol) in 0.5 ml of DMSO. The reaction mixture was stirred at room temperature for 24 hours, a precipitate being formed. After the addition of 1 ml of water, the precipitate was separated off by centrifugation, suspended three times in 1 ml of MeOH each time and again centrifuged. After drying *in vacuo*, 0.5 ml of trifluoroacetic acid was added to the residue. After two hours, 3 ml of toluene were twice added and the solution concentrated. The residue was dissolved in water and, after the addition of 0.1 ml of a 2M HCl solution, evaporated to dryness. The product was obtained by means of gel chromatography over a Sephadex LH-20 column (1×30 cm) with a 50 % aqueous acetonitrile solution. After freeze-drying of the product fraction, 26 mg (63 %) of compound (**52**) were obtained.

^{1}H-NMR spectrum in D_2O (δ, ppm): 1.455 (m, 4H, $CH_2C\underline{H}_2C\underline{H}_2CH_2$), 3.172 (m, 4H, $2CH_2N$), 3.856, 3.872, 3.947, 3.960, 3.975 and 4.028 (s, 2H, $COCH_2N$).

The aggregate *{[Neu5Acα-OCH$_2$(p-C$_6$H$_4$)NHCOCH$_2$NH-CO(CH$_2$)$_4$CO-(NHCH$_2$CO)$_6$-NHCH$_2$CH$_2$-]$_2$}$_x$ (ammonium salt)* **{[Neu5Ac-Gab-Ad-Gly$_6$-NHCH$_2$CH$_2$-]$_2$}$_x$ (53)** was prepared analogously to compound (**48**) in Example 7.
Yield 72 %
^{1}H-NMR spectrum in D_2O (δ, ppm): matrix: 1.470 (m, 4H, $CH_2C\underline{H}_2C\underline{H}_2CH_2$), 1.649 (m, 4H, $COCH_2C\underline{H}_2$), 2.363 (m, 4H, $2COC\underline{H}_2CH_2$), 3.181 (m, 4H, $2CH_2N$), 3.869, 3.941, 3.962, 3.977 (x3) and 4.045 (s, 2H, $COCH_2N$), 4.505 and 4.727 (d, 2H, J 11 Hz, $ArCH_2$), 7.415 (m, 4H, Ar). Neu5Acα fragment : 1.680 (dd, 1H, J_4 12 Hz, H-3ax), 2.086 (s, 3H, NAc), 2.778 (dd, 1H, J_{3ax} 12.5 Hz, J_4 4.6 Hz, H-3eq), 3.598 (dd, 1H, J_8 9 Hz, H-7), 3.636 (dd, 1H, J_8 6 Hz, H-9b), 3.695 (ddd, 1H, J_5 9.8 Hz, H-4), 3.728 (dd, 1H, J_7 1.5 Hz, J_5 10.2 Hz, H-6), 3.782 (ddd, 1H, H-8), 3.822 (dd, 1H, H-5), 3.846 (dd, 1H, J_{9b} 12 Hz, J_8 2.3 Hz, H-9a).

Example 13

Preparation of NeuAcα2-6Galβ1-4GlcNAcβ-O$(CH_2)_3$NH-CO$(CH_2)_4$COO(p-$C_6H_4NO_2$) **6'SLN-Ap-Ad-ONp (52).**

A solution of 65 mg (195 µmol) of di-(4-nitrophenyl) adipate (3) in 300 µl of DMF was added to a solution of 28 mg (38 µmol) of the compound 6'SLN-O$(CH_2)_3NH_2$ in 400 µl of DMSO. The mixture was stirred at 20°C for 16 hours. After the addition of 5 ml of H_2O and 0.1 ml of AcOH, the excess (3) was filtered off. The filtrate was concentrated to a small volume of about 1 ml and separated by means of gel permeation chromatography over Sephadex LH-20 (MeCN /H_2O/AcOH 1:1:0.005).
Yield (52) - 71%. TLC: R_f 0.46 (i-PrOH / acetone /H_2O 4:3:2).
^{1}H-NMR spectrum (D_2O, δ, ppm): 1.641 (m, 6H, 2 $COCH_2C\underline{H_2}$ and $OCH_2C\underline{H_2}$), 1.674 (dd, 1H, H-3-ax Neu5Ac), 1.930 and 1.958 (s, 2x3H, 2 $COCH_3$, Neu5Ac and GlcNAc), 2.218 (t, 2H, $NCOC\underline{H_2}$), 2.559 (dd, 1H, J_{3ax}, 13Hz, J_4 4.7 Hz, H-3eq Neu5Ac), 2.646 (m, 2H, CH_2COOAr), 3.090 and 3.190 (m, 2x1H, NCH_2), 3.42-3.94 (21H, overlapping of the carbohydrate signals and OCH_2), 4.328 (d, 1H, J_2 8 Hz, H-1 Gal), 4.419 (d, 1H, J_2 8 Hz, H-1 GlcNAc), 7.291 and 8.256 (d, 2x2H, J 8.3 Hz, Ar).

Preparation of **[6'SLN-Ap-Ad-Gly_7-$NHCH_2$]$_4$C (53)**

15 mg (16.2 µmol) of the compound NeuAcα2-6Galβ1-4GlcNAcβ-O$(CH_2)_3$NH-CO$(CH_2)_4$COO(p-$C_6H_4NO_2$) (52) were added to a solution of 5 mg (2.7 µmol) of the tetrahydrochloride [HCl Gly_7-$NHCH_2$-]$_4$C (22a) in 500 µl of H_2O.
The pH value of the resulting solution was adjusted to pH~8 by the dropwise addition of 1M $NaHCO_3$. The reaction mixture was stirred at room temperature for 3 days and separated by means of gel permeation chromatography (G10, 0.05M NH_3).
Yield (53) 34%, TLC: R_f~0 (i-PrOH /acetone/H_2O 4:3:2).
^{1}H-NMR spectrum (D_2O, δ, ppm): matrix: 1.628 (m, 4H, $COCH_2C\underline{H_2}$), 1.789 (m, 2H, $OCH_2C\underline{H_2}$), 2.275 and 2.373 (m, 2x2H, 2 $COC\underline{H_2}CH_2$), 2.935 (s, 2H, CCH_2), 3.197 and 3.279 (m, 2x1H, NCH_2), 3.971, 3.990, 4.026 (x3) and 4.077 (x2) (s, 14H, 7 CH_2^{Gly1-7}). Carbohydrate signals: 1.730 (dd, 1H, H-3ax Neu5Ac), 2.049 and 2.078 (s, 2x3H, 2 $COCH_3$, Neu5Ac and GlcNAc), 2.693 (dd, 1H, J_{3ax} 12.4 Hz, J_4 4.6 Hz, H-3eq Neu5Ac), 3.54-3.96 (21 H, overlapping of the carbohydrate signals and OCH_2), 4.468 (d, 1H, J_2 8Hz, H-1 Gal), 4.562 (d, 1H, J_2 8 Hz, H-1 GlcNAc).

The compound **[6'SLN-Ap-Ad-AC_2-Gly_5-$NHCH_2]_4C$ (54)** was prepared in an analogous manner starting from the tetrahydrochloride [HCl·H-AC_2-Gly_5-$NHCH_2]_4C$ (24a) and NeuAcα2-6Galβ1-4GlcNAcβ-$O(CH_2)_3NH$-$CO(CH_2)4COO(p$-$C_6H_4NO_2)$ (52).
Yield (54) - 63%. TLC: R_f~0 (i-PrOH/acetone/H_2O 4:3:2).

^{1}H-NMR spectrum in D_2O (δ, ppm): matrix: 1.341, 1.524 and 1.631 (m, 12H, 6 CH_2), 1.599 (m, 4H, $COCH_2C\underline{H_2}C\underline{H_2}CH_2CO$), 1.785 (m, 2H, $OCH_2C\underline{H_2}$), 2.238 (t, 2H, J 7.4 Hz, CH_2CO), 2.260 (m, 4H, $COC\underline{H_2}CH_2CH_2C\underline{H_2}CO$), 2.349 (t, 2H, J 7.5 Hz, CH_2CO), 2.929 (s, 2H, CCH_2), 3.182 (broad t, 4H, J 6.6 Hz, 2 CH_2N), 3.195 and 3.275 (m, 2x1H, NCH_2), 3.979, 4.022 (x3) and 4.073 (s, 10H, 5 $COCH_2N$). Carbohydrate signals: see (53).

Table 9, Example 13
Inhibition of the viral time-adhesion of influenza viruses; strain A/NIB/H1N1/89M, FBI text [see Table 1], 6'SLN as reference compound.

Inhibitor	Compound No.	Relative activity
6'SLN		1
[Neu5Ac-Gab-Ad-Gly_7-$NHCH_2$-$]_4C$	(48)	< 0.2
[6'SLN-Ap-Ad-Gly_7-$NHCH_2]_4C$	(53)	100
[6'SLN-Ap-Ad-AC_2-Gly_5-$NHCH_2]_4C$	(54)	1000

Comparison Example 1

The compound of formula
{Neu5Acα2-6Galβ1-4GlcNAcβ1-$NHCOCH_2NH$-$CO(CH_2)_4CO$-$(NHCH_2CO)_3$-$NHCH_2$-$\}_4C$,
which is known from Example 7 of WO 98/14215, does not form aggregates according to the invention, as has been demonstrated by the following methods:

1. In thin-layer chromatography only a single compound, the monomer, can be observed under the following conditions. Traces of aggregates are completely absent:

silica gel 60 TLC plates, catalogue no. 1.05724, Merck; eluant: *i*-PrOH / acetone / H_2O 4:3:2; observation: charring after immersion in 7% H_3PO_4.

2. The ^{1}H-NMR spectrum (Bruker 500 MHz, D_2O, 300 K) of the compound does not exhibit any line broadening that would be characteristic of glycopeptide aggregates.

3. Laser light-scattering experiments (Coulter Submicron Model N4MD, He-Ne laser, I 632.8) on the compound in aqueous solution do not give any indication of the formation of aggregates.

4. Using gel permeation HPLC (TSK-4000 column, 0.2M NaCl), only one peak is observed, which corresponds to the molecular weight of the monomer.

5. The activity of the compound in the inhibition of influenza viruses (A/NIB/23/89M H1N1, A/NIB/44/90M H3N2, B/NIB/15/89M, FBI test) is comparable with that of 6`SLN trisaccharide, whereas typical associated glycopeptides, such as, for example, the Gly_7 analogon) are more active by factors of ten.

It is therefore clear that the compound known from the prior art is fundamentally different from the compounds of the present invention. The demonstrated functional difference is based on the structural difference of the fragment K, which is not suitable for intermolecular association because the number of chain segments suitable for the formation of hydrogen bridges is too small.